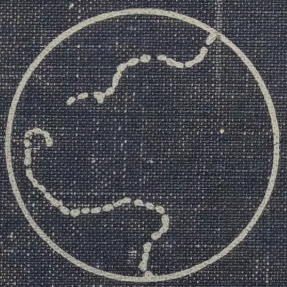
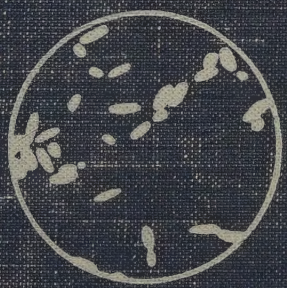


Dairy Bacteriology

4th edition



BERNARD W. HAMMER

FREDERICK J. BABEL

Digitized by the Internet Archive
in 2025

https://archive.org/details/dairybacteriolog0000bern_m3x9

Dairy Bacteriology

NEW YORK · JOHN WILEY & SONS, INC.

London · Chapman & Hall, Limited

Dairy Bacteriology

4TH EDITION

BERNARD W. HAMMER, Ph.D., D.Agr.

FREDERICK J. BABEL, Ph.D.

Professor of Dairy Bacteriology

Purdue University

Copyright, 1928, 1938, 1948, © 1957 by John Wiley & Sons, Inc.

All rights reserved. This book or any part thereof must not be reproduced in any form without the written permission of the publisher.

Library of Congress Catalog Card Number: 57–10806
Printed in the United States of America

637
H22d
1957

Preface to Fourth Edition

The fourth edition of *Dairy Bacteriology* covers the same general material as the earlier editions and, again, the expansion or contraction of certain sections has been based on the increasing or decreasing importance of the subjects involved. Extensive work on antibiotics and bacteriophage has resulted in considerable increases in the sections covering these fields. In general, the first ten chapters cover material of importance to both the producer of milk and the manufacturer of dairy products while the last ten chapters concern primarily the bacteriology of manufactured products.

As in the earlier editions, only special laboratory methods are included, and *Standard Methods for the Examination of Dairy Products* (last edition) should be used for the procedures given there. The changes that have been made in methods which at one time were thought to be thoroughly standardized show that the last edition of a publication giving a laboratory procedure should be followed exactly.

In the fourth edition, for the first time, the changes were determined by the ideas of two authors instead of only one.

In the general field of dairy bacteriology numerous important problems need solution. The control of objectionable numbers and types of organisms in dairy products often is simple, and this suggests that all the problems in dairy bacteriology are in the same category. Actually, many problems along such lines as development of the desired flavors in certain products through the action of organisms or economic

production of various fermentation materials from milk solids that would otherwise be wasted are complicated, and the practical solution of any one of them constitutes a real accomplishment.

The names of the authors commonly are used in connection with the studies reported because an adequate understanding of dairy bacteriology requires some knowledge of the investigators who have worked in the various fields.

In the revision, suggestions have come from a number of individuals; all such help is appreciated greatly.

Sarasota, Florida
West Lafayette, Indiana

B. W. Hammer
F. J. Babel

Preface to First Edition

The material presented in the following pages represents that included in the beginning course in dairy bacteriology at Iowa State College. This course follows one in general bacteriology and is intended primarily for students in the course in dairy industry but is also required of students in dairy husbandry and is elected by others specializing in bacteriology. Four recitation-lecture periods are held each week for one quarter of about twelve weeks. Assigned readings intended to supplement the material presented and to give the students a more detailed knowledge of important points than the class room permits are also required. The laboratory work consists of three two-hour periods each week and the exercises are selected with the idea of illustrating the more important procedures used in dairy bacteriology. Considerable time is spent on the milk fermentations since a good understanding of these is believed to be fundamental to the practical knowledge of dairy bacteriology which is so essential to the technical dairyman. Some of the organisms studied are isolated by the students themselves from milk showing various changes. This illustrates the distribution of the organisms studied better than can be done in any other way and also adds interest to the work; the continued study of supplied cultures tends to give the students the impression that bacteria exist mainly in laboratory cultures.

The relative importance, from the standpoint of the class room, of the various phases of dairy bacteriology is a question on which con-

siderable diversity of opinion is to be expected. The proportion maintained in the following presentation has been found to be satisfactory under the conditions existing at Iowa State College and satisfactorily to relate dairy bacteriology to the courses that follow it.

Material on the standard methods for the bacteriological examination of milk is not included because it is so readily available elsewhere in a form adapted to laboratory use. For the most part, descriptions of organisms are omitted because brief descriptions are included in books dealing with determinative bacteriology and detailed descriptions can be best studied in the original reports.

B. W. Hammer

Dairy Industry Building
Ames, Iowa

Contents

INTRODUCTION 1

1 BACTERIAL COUNTS ON DAIRY PRODUCTS 3
2 TESTS FOR THE GENERAL QUALITY OF RAW MILK 26
3 MILK FERMENTATIONS 63
4 BACTERIOLOGY OF MILK PRODUCTION 116
5 WASHING, BACTERICIDAL, AND PRESERVING REAGENTS 156
6 GROWTH OF BACTERIA IN RAW MILK 174
7 MILK PLANT PROCESSES 189
8 MILK PLANT OPERATION 234
9 PATHOGENS IN DAIRY PRODUCTS 252
10 MILK ENZYMES 294
11 BACTERIOLOGY OF EVAPORATED MILK 314
12 BACTERIOLOGY OF SWEETENED CONDENSED MILK 327
13 BACTERIOLOGY OF MILK POWDER 336
14 FREEZING MILK AND CREAM 347
15 BACTERIOLOGY OF ICE CREAM 353
16 BACTERIOLOGY OF LACTIC CULTURES 373
17 BACTERIOLOGY OF FERMENTED MILK PREPARATIONS 431
18 BACTERIOLOGY OF BUTTER 442
19 BACTERIOLOGY OF CHEESES 510
20 BACTERIOLOGY OF DAIRY-PLANT WATER SUPPLIES 593

INDEX 603

Introduction

IMPORTANCE OF DAIRY BACTERIOLOGY

The need for knowledge of the bacteriology of dairy products is shown by four distinct considerations:

1. Pathogens in Dairy Products. Investigations extending over many years have proved that diseases in both man and animals sometimes are caused by pathogens spread through milk and its derivatives. Accordingly, knowledge of the sources of pathogens in dairy products, conditions under which they grow, and methods of keeping them out or destroying them when they gain entrance is essential. Such knowledge is important to the distributor of dairy products, because of the responsibility of selling only safe foods, and it is of extreme importance to the inspector of dairy plants and dairy products since inspection is intended to represent the final protection to the consumer. Although the distributor and the inspector should guard the consumer's interests, it is desirable that the consumer be sufficiently informed to decide whether a particular system of handling a dairy product is satisfactory. In the same way, anyone using milk or milk products for the feeding of animals should have the knowledge that will make possible a reliable decision as to the probability of such materials introducing diseases into herds and flocks.

2. Deterioration and Spoilage of Milk and Its Derivatives. Many micro-organisms, as a result of their growth, cause undesirable changes in milk and its derivatives and, accordingly, are responsible for deterioration and spoilage. The producer of milk and cream should know the sources of organisms causing rapid changes in these materials, conditions favoring their growth, and methods of eliminating them or preventing their activity, since the price depends, in part, on the quality of the products supplied. The manufacturer of milk derivatives must contend with problems similar to those of the producer of milk and cream and additional ones also, since butter, cheeses, etc., frequently are stored for considerable periods, during which there may be opportunities for a decrease in quality. The problem of deterioration and spoilage is especially important with the cheeses requiring ripening, since conditions must be favorable for growth of certain desirable micro-organisms and these may allow development of undesirable species.

3. Development of Desirable Micro-organisms in Certain Dairy Products. The pleasing flavor of some dairy products, such as cheeses, fermented milk, and some types of butter, and the desired texture of many cheeses are due largely to the development of certain micro-organisms during manufacture and/or ripening. An understanding of the action of these organisms, conditions favoring their growth, etc., is important and very necessary if products having a uniformly high quality are to be produced. The manufacturing methods used for certain dairy products were developed empirically before the role of micro-organisms was understood, but various valuable improvements have resulted from knowledge of the action of the important species and progress along this line undoubtedly will continue.

4. Sanitary Regulations. The sanitary regulations covering milk and various derivatives of it are based, to a considerable extent, on the bacteriology of these products. Accordingly, an understanding of the regulations and their significance is simplified for the producer and distributor of dairy products by a knowledge of the bacteria in these materials.

1 Bacterial Counts on Dairy Products

Bacterial counts, or estimates, are used extensively in the examination of dairy products. In the grading of milk and in the general control of milk supplies, *routine* bacterial counts are employed. With them, the results must be obtained cheaply and quickly. Standardized methods are necessary with routine counts so that results obtained in different laboratories, or in one laboratory at different times, can be compared and the significance of the counts interpreted on the basis of general experience in milk control work. It is not necessary that a routine count give the actual number of bacteria in a sample but rather that a representative portion be counted. The bacterial limits set by ordinances and regulations recognize that routine methods do not necessarily give as high counts as might be obtained with special procedures.

In investigations of the normal changes in such products as cheeses, lactic cultures, butter, etc., and in studies on spoilage in various dairy products, *special* methods of counting bacteria are needed. Often these involve primarily the counting of the numbers of certain groups or types of organisms. With such counts accuracy under specific conditions is the principal consideration, and expense and period required are not important factors.

ROUTINE COUNTS

Two methods commonly are employed in the routine counting of the bacteria in milk: the microscopic count, or direct microscopic method; and the agar plate method. The procedures used with these methods have been well standardized for use in the supervision of milk supplies.[1] Each has been changed from time to time, and it is essential that the current procedures be followed because of the influence of various factors on the results.

Microscopic Count. Methods of counting bacteria in various materials by means of a microscope have been employed since the early days of bacteriology, and eventually the idea was applied to milk. The method now used involves spreading 0.01 ml of milk, measured with a special syringe or capillary pipette, over an area of 1 sq cm on a glass slide and, after the milk is dried and the fat removed, the staining and examination of the resulting film. From the bacteria counted in a number of microscopic fields, the bacterial count per 1 ml of milk is calculated.[1] Counts can be made on two bases: (1) individual cells and (2) clumps of cells in which unseparated groups, chains, pairs of cells, and isolated cells are counted as units. The second procedure attempts to make microscopic counts more nearly comparable to plate counts in which colonies often come from groups of cells. Black [2] found that the requirements of the method were violated rather frequently by milk laboratories in the United States.

For special screening or field tests, the preparation of slides for counting can be speeded up by using a standardized loop to measure the milk and a combined fat solvent and staining solution (Newman-Lampert stain) to treat the milk film.[1]

Advantages of Microscopic Count. The microscopic count has certain advantages over the plate count, the more important of which are:

1. Results Are Quickly Available. With the microscopic method a count can be obtained in a very short period, provided the microscope has been standardized and the reagents prepared; this is particularly advantageous in connection with samples brought to laboratories by producers, since such persons usually wish the results at once and often are interested in seeing the bacteria in the milk.

2. Less Work Is Required. The number of samples that can be examined in a given period with the microscopic method is relatively large, owing mainly to the fact that preparation of the agar and dilu-

tion bottles, washing and sterilization of the glassware, etc., are not involved. The actual number is influenced primarily by the number of fields counted per sample. If milk shipments are simply divided into grades, as in certain types of inspection work, they can be handled very rapidly; high-count samples often can be graded by examination of relatively few fields.

3. Less Apparatus Is Required. The amount of apparatus necessary for the microscopic count is relatively small, a microscope being the only expensive item. With the plate method the investment in incubators, sterilizers, and glassware is comparatively large.

4. Less Expensive. Since the microscopic count requires less work and apparatus than the plate method, expense per sample also is less, especially because of the cost and maintenance of equipment for the plate method. If bacterial counts are to be used extensively, the lowest possible cost is advisable because the funds available often are limited. With only a small number of bacterial counts on a given milk supply, the conclusions drawn may be open to criticism.

5. Gives a Permanent Record. The preparations used in making microscopic counts can be filed and examined whenever, within a reasonable period, a question arises with reference to them. Permanent records are especially important in connection with certain points, such as morphology of organisms, that are difficult to report and not easily included in a statement of results.

6. Gives Some Idea of Types of Organisms Present. Although no definite information regarding species can be obtained from microscopic observations alone, some idea of the general types of bacteria present can be secured by persons familiar with the organisms common in milk. A predominance of the ordinary lactic acid bacteria is determined rather easily from microscopic preparations, and some of the organisms from infected udders can be recognized with considerable accuracy. Information on the types of organisms frequently is valuable in helping determine the cause of high counts on certain samples.

7. Numbers of Body Cells Present Can Be Determined. The stained preparations employed in the microscopic method permit the counting of leucocytes and other body cells in milk, and unusually large numbers are evident from superficial examination. Although the significance of these cells, even in rather large numbers, may be questioned, excessive counts indicate abnormal udder conditions, particularly when associated with large numbers of streptococci. The

analyst experienced in the microscopic method frequently can detect abnormal udders by the microscopic picture of the milk.

8. Preservative Can Be Used with Samples. Milk samples intended for microscopic examination can be held for short periods with formaldehyde because the bacteria need not be kept in a living condition. Samples so treated cannot be held for long, however, since the organisms killed by the preservative soon begin to autolyze and this interferes with their staining. Formaldehyde in a concentration of 0.08% apparently preserves milk satisfactorily for 3 days.[3, 4]

Disadvantages of Microscopic Count. The microscopic count also has disadvantages compared to the plate method. The most serious of these are:

1. Irregularities Are Inherent in the Procedure. Results obtained with the microscopic count are influenced by the irregularities inherent in it. Organisms often are unevenly distributed in a smear. In some smears many fields show few or no bacteria but an occasional field shows many cells; under these conditions the results are influenced by the number of high-count fields that happen to be encountered. The milk equivalent of the portion of the smear examined is relatively small and may not be representative of the sample. Smears vary in thickness so that a field does not always represent exactly the same volume of the original milk. There is considerable evidence that not all the bacteria in a smear stain well enough to permit their detection. Some bacteria that do stain may not be recognized as organisms, especially with low magnification. Because of these irregularities, there may be considerable variation between duplicate counts on a sample.

2. Less Generally Useful for Low-count Milk. Irregular distribution of organisms in a smear is especially significant with low-count milk. The microscopic count can be used with such milk, but it is a laborious procedure if reasonably detailed results are desired; simple detection of low-count milk is not particularly time-consuming.

3. Less Generally Useful for Pasteurized Milk. When a microscopic count is made on pasteurized milk a portion of the organisms that have been killed by the heat still take the stain. This limits the usefulness of the method in supervision of city supplies and in checking operations in pasteurizing plants. It appears that the percentage of killed cells which can be seen varies with the species present and also with the care used in making the observations, etc.

Hastings and Davenport [5] noted that as a result of pasteurization at 145 F for 30 min there was a decrease in the number of bacteria detected by the microscopic method which, in some instances, was very

large. The decrease often was more after 6 hr at 50 F than immediately after heating and, occasionally, there was a further decrease after 24 hr. Pasteurization of low-count milk inoculated with pure cultures commonly gave results similar to naturally contaminated milk, although with some species there was no apparent change in the staining or number of cells. Watrous and Doan [6] found no significant relationship between the microscopic clump count and plate count of the organisms in fresh laboratory-pasteurized milk. Nonviable organisms were capable of stain retention to a varying degree, depending on the flora of the milk. Microscopic clump counts on fresh laboratory-pasteurized milk were lower than counts prior to pasteurization, indicating that not all of the cells destroyed by pasteurization are stained.

Hill [7] made microscopic counts on unheated milk and portions of the same milk heated for 30 min each at temperatures from 122 to 212 F. The counts were made either immediately after heating or after 24 hr at 41 F. The numbers of stainable organisms decreased as the temperature increased, this effect being more marked in the samples counted after holding. The shadowy forms, which were observed in all the heated samples, tended to decrease in number with increasing temperatures. No relationship was found between the number of shadowy forms present and the number of bacteria killed.

In some cases attempts are made to distinguish between living and dead bacteria in smears of pasteurized milk by the intensity of the staining. However, there are variations in the staining intensities of the organisms in smears from raw milk so that conclusions are open to question. Stark and Campbell [8] noted that with the microscopic count it is not always possible to distinguish with certainty between living and dead bacteria.

Agar Plate Method. The introduction of liquefiable solid media afforded a means of determining the numbers of bacteria in various materials by mixing a given weight (or volume) with the liquefied and cooled medium in plates, allowing this to solidify, counting the colonies that develop on incubation, and then calculating the count to the desired basis. The procedure has been used widely in the control of milk supplies.

The medium used for plating milk, temperature and period of incubation, and other variations in the procedure have an influence on the numbers of colonies developing through their general effect on growth of organisms. Black [2] surveyed milk laboratories in the United States and found that with agar plate counts each item required by

standard methods had been adhered to by several laboratories but that practically every item had been neglected by one or more laboratories. At the time of the survey not one laboratory actually met all requirements for equipment and procedure, although a very few approached them.

Advantages of Plate Method. The plate method is applied readily to the types of milk with which the microscopic count is less generally useful.

1. Low-count Milk. The bacterial counts of milk have been greatly reduced through better methods of production and much of the milk now reaching plants has so few bacteria that the microscopic count is not readily applied to it. Moreover, with the low counts, relatively few plates need to be poured to obtain satisfactory results, so the plate method is simplified.

2. Pasteurized Milk. The wide use of pasteurization in the milk industry has resulted in many samples of milk with which the desired information is most readily obtained with the plate method.

Objections to Plate Method. Objections have been raised to the plate method of determining the numbers of bacteria in milk as a means of controlling milk supplies. The more important of these are:

1. Counts Are Not Accurate. Counts obtained by the plate method do not represent the actual numbers of bacteria present because of various factors. Certain organisms in milk do not grow on the plates because the nutrients, air supply, or temperature are not satisfactory. Many of the colonies come from pairs or groups of cells rather than one cell. Accordingly, the values obtained with the plate method are lower than the actual numbers of bacteria present; extent of the discrepancy is dependent mainly on the species in the milk. The fact that the plate count is lower than the actual number of bacteria in a sample of milk is of no great consequence in control of milk supplies since an allowance is made for this in the establishment of bacterial standards; however, the method used should give a fairly constant percentage of the total numbers present.

In some instances large discrepancies [9,10,11] have occurred in results obtained by various laboratories on milk from the same lot. These may have been due to variations in the media used, in the periods between diluting the milk and pouring the plates, in the methods of incubating the plates, and in other factors, all of which are important. Differences in the results obtained by persons working in the same laboratory also have been noted.

Tiedeman [12] reported that standard plate counts made by different

laboratories on identical samples of low-count raw milk are in close agreement; such counts on high-count raw milk, abnormal raw milk, and pasteurized milk show marked variations. He also noted that the preponderance of standard plate counts falling out of range is on the low side, suggesting failure of some of the bacteria to produce discernible colonies.

2. Specific Information Regarding Species Is Not Furnished. With most organisms the colony appearance does not identify the species. If the organisms in a sample of milk could be classified from the colonies that develop on agar, valuable information would be available. However, failure to obtain definite information regarding species cannot be considered a serious objection to the plate method since the counts are made primarily to determine the general bacteriological quality of the milk.

3. Period Required before Results Are Available Is Too Long. Because of the period required for incubation of the plates, data on the number of bacteria in a sample of milk ordinarily are not available until the milk has been consumed. The quality of a milk supply, however, cannot be determined by one bacterial count, and it is only when results of a series of counts are available that a fair idea of the character of a supply can be formed. A single high count does not justify exclusion of the lot of milk on which it was obtained if the counts on the preceding lots from the same source are satisfactory; it simply indicates an undesirable condition that should be located and corrected at the earliest possible moment. When the real significance of a single high count is recognized, it is evident that consumption of milk before the count on it is available does not constitute an important objection.

Comparison of Microscopic and Plate Counts. Bacterial standards for various grades of milk generally are based on microscopic clump counts or plate counts, the same standards applying with either method.[13] This suggests that the ratio of the results of the two methods is 1 to 1. Watrous and Doan,[6] using a special counting technic, obtained a ratio approaching 1 to 1 on samples of raw milk when the plate and microscopic clump counts were compared. Some variation is to be expected in this ratio when comparisons are made on samples having widely different counts. For example, Black [14] obtained slightly higher plate counts than microscopic clump counts on poor-quality raw milk and slightly higher microscopic clump counts than plate counts on good-quality milk. These results may be explained, in part, by the fact that in high-count milk the organisms are largely those that

have grown in milk, many of which are capable of developing on the agar used for plating, whereas in low-count milk the organisms are mainly those that have come from various sources and include types that will not grow on the agar.

For certain purposes, a count of the individual cells in a sample may be preferred. With this procedure it often is necessary to interpret the results in terms of the clump count or plate count. Robertson [15] found the most frequent ratio between results of microscopic (individual cell) and plate counts, when the plates were incubated 2 days at 98.6 F (37 C), was 4 to 1; it was 2.5 to 1 when the plates were incubated 5 days at 69.8 F (21 C). The ratio commonly recognized, and the one used when it is necessary to calculate results of individual cell microscopic counts in terms of microscopic clump or plate counts, is 3.33 to 1.[1] Actually, the ratio varies widely from one lot of milk to another and is influenced by the species present and other factors. Baker *et al.*,[16] in their study of the relation between lactic acid production and bacterial growth in the souring of milk, noted that the average size of the groups of lactic acid organisms in milk was 1.8 individuals. Whiting [17] found the average size of the clumps of bacteria in 3,480 samples of market milk from Geneva, N. Y., was 11.1 individuals, while in 150 samples from Ithaca, N. Y., it was 4.1 individuals; the word *clump* was used to refer to isolated cells as well as to groups.

Significance of Routine Counts on Raw Milk. Routine bacterial counts on raw milk give information along four lines as follows:

1. Care Used in Production. The care used in production of milk, especially with reference to such factors as condition of equipment and cleanliness of the coats of the cows, is reflected in the bacterial count of the freshly drawn milk. However, even with very insanitary methods of production the counts are not surprisingly high when the milk is first drawn; they rarely exceed 1,000,000 per ml and commonly fall below 500,000. It is multiplication of organisms gaining entrance to the milk, rather than contamination, that is responsible for the very high counts sometimes obtained.

2. Temperature of Holding. The rate of multiplication of bacteria in milk is influenced more by the temperature of holding than by any other factor. At favorable temperatures growth is surprisingly rapid and excessive numbers of organisms quickly develop. Very high bacterial counts on milk more often are due to holding temperatures that are especially favorable for multiplication than to any other cause.

3. Period of Holding. The period of holding is another important factor influencing multiplication of bacteria in milk and one that naturally is related to the temperature of holding. As short a period as 1 hr may account for an increase in the number of bacteria in milk if the holding temperature is very suitable for growth, whereas longer periods are required for significant increases as the temperature becomes less favorable. Temperatures that satisfactorily check bacterial development for 24 hr may not do so over more extended periods. If milk is held long enough, multiplication of bacteria occurs at all the usual temperatures above the freezing point.

4. Condition of the Udder. Rather high bacterial counts on milk may be obtained as a result of the presence of large numbers of bacteria in the milk at the time it leaves the udder. Frequently, such milk comes from infected udders, but it also may come from udders that apparently are normal. There is no close correlation between infected udders and rather high counts on milk as it is drawn, and in some instances milk coming from such udders has an unusually low bacterial count.

The microscopic pictures shown by preparations for microscopic counts on milk are more useful than plate counts in detecting infected udders. Morgan *et al.*[18] concluded that the conditions under which milk is produced and handled have a much greater influence on the plate count than does the incidence of *Streptococcus agalactiae* (the common cause of mastitis in cows) infection in the animals. In some cases S. *agalactiae* was responsible for a large proportion of the plate count on low-count milk but for only a small proportion of the plate count on high-count milk. Moore[19] made plate counts on aseptically drawn milk from a herd of 95 cows, about half of which were known to be infected with S. *agalactiae* in at least one quarter. The presence of S. *agalactiae* in milk did not permit predicting whether the plate count would be high or low.

Information given by routine bacterial counts along the four lines considered is suggestive rather than definite. When a high count is obtained it usually is more or less difficult to decide which of the various factors is responsible, although laboratory experience often makes it possible to give a reasonably accurate opinion. A variety of bacterial types suggests extensive contamination, many colonies of the common lactic acid organisms indicate growth at temperatures higher than those advocated for holding milk, etc. Frequently, high counts are due to a combination of factors, and determination of the cause

then is more complicated than when only one factor is involved. Although the history of a sample of milk, as determined by the number of contained bacteria, is not as definite as is desired, defective methods of production and handling often can be detected and, accordingly, bacterial counts are indispensable in the proper control of milk supplies.

The relatively low cost of bacterial counts is an important consideration and it generally is recognized that, for the expense involved, bacterial counts give more information regarding methods of producing and handling milk than any other procedure. Bacterial counts are employed extensively by milk distributors as well as health departments and other supervising agencies. A bacteriological laboratory is an important part of most modern dairy plants and often large numbers of bacterial counts are made on the milk as it comes from the producers; in certain instances a premium is paid for milk of high sanitary quality, as shown by these counts or other tests.

Significance of Routine Counts on Pasteurized Milk. When applied to pasteurized milk, plate and microscopic counts give information along somewhat different lines.

In the supervision of supplies of pasteurized milk, plate counts aid materially in controlling the efficiency of both the pasteurization and the handling of the milk after heating. Recording thermometer charts are important, but there is no assurance that all the milk was heated under the recorded conditions and, moreover, the value of efficient pasteurization may be counterbalanced by careless handling subsequent to this treatment. In some cases also the raw milk contains large numbers of heat-resistant organisms that can best be detected with plate counts.

Even with the irregularities in the staining of the bacteria in pasteurized milk, microscopic counts supply valuable information with reference to the numbers of bacteria present before heating and also help to explain unsatisfactory pasteurization by showing the general morphologic types of bacteria present.

In conjunction with other tests, microscopic counts on pasteurized milk are of increasing importance.[20, 21] A program of (1) phosphatase tests to determine adequate heating of all the milk, (2) tests for coliform organisms to detect contamination of the heated milk, and (3) microscopic counts to establish the bacteriological quality of the original milk provides a relatively simple means of controlling a milk supply.

Bacterial Standards. Recognition of the relationship between the bacterial counts on milk and the care used in its production and handling resulted in the establishment of bacterial standards for market milk. In 1900, the New York City Department of Health attempted a standard of 1,000,000 per ml, but it was found practically impossible to enforce at that time because of the poor bacteriological quality of the milk. In 1905, the Boston Board of Health legalized a standard of 500,000 per ml. Since then many areas have established bacterial standards for different grades of milk and included various other requirements covering the production and handling of the product. The bacterial standard for cream normally is higher than that for milk of the same grade since, during skimming, considerable numbers of bacteria may be carried into the cream by the clumps of fat globules. On pasteurized products counts of coliform organisms made with a special medium (see p. 19) often are included in the standards since more than a very limited number of these organisms indicates contamination subsequent to the heating.

Recently, there has been a marked tendency toward uniformity in standards, and many states and cities have adopted the standards set forth in the Milk Ordinance and Code of the U. S. Public Health Service.[13] These are shown in the following summary.

Table 1. Summary of Bacterial Standards for Market Milk and Cream from Ordinance and Code U.S.P.H.S.

	Bacteria per Milliliter, Not to Exceed		Coliform Organisms per Milliliter, Not to Exceed	
Type of Milk or Cream	When Raw	When Pasteurized	When Raw	When Pasteurized
Raw certified milk	10,000		10	
Pasteurized certified milk	10,000	500	10	1
Raw Grade A milk	50,000			
Pasteurized Grade A milk	200,000	30,000		10
Pasteurized Grade A cream	400,000	60,000		10
Pasteurized Grade B milk	1,000,000	50,000		10
Pasteurized Grade B cream	2,000,000	100,000		10
Pasteurized Grade C milk	*	*		*
Pasteurized Grade C cream	*	*		*

* No limit.

SPECIAL COUNTS

Both microscopic and plate counts are used widely in investigations on dairy products, the one that is adopted depending primarily on the main objective. Often the two methods are used synchronously since microscopic counts aid in determining whether all the morphologic types of organisms that are numerous in the material being studied are developing on the plates. In some instances bacteria that were abundant in a product have been missed entirely because they failed to form colonies under the conditions provided.

Counts made by the microscopic method also may need to be verified. Whitehead [22] noted that, when organisms grown for 24 hr on an agar medium were added to milk and microscopic counts made, results with certain species were satisfactory, whereas with other species it rarely was possible to detect the added organisms. It appeared that some species did not acquire staining properties sufficient to render them visible until they had begun to multiply in the milk.

In employing the plate method in investigational work, it usually is desirable to have the counts as accurate as possible and, accordingly, special media and incubation conditions commonly are used. When some particular group of organisms is being studied, a medium and an incubation temperature that are especially favorable can be adopted.

Media for Plate Counts. Media used for routine plate counts on milk are satisfactory for many of the organisms present in dairy products and have been used extensively for special counts. Ordinary nutrient agar is much less satisfactory, and simple modifications of it have been suggested. Sherman [23] reported that addition of lactose increased both the number and size of the colonies developing on plates poured with milk and incubated 48 hr at 98.6 F (37 C). Other investigators have noted this same effect when different sugars were added to various media.

Tryptone glucose extract (milk) agar, which formerly was the standard medium for routine counts on milk, has been employed extensively for special counts. Beef infusion agar also is very useful; with it, colonies often are quite large. Tomato agar has been employed in studying the acid-producing organisms in sour milk and cream, lactic culture, butter, and various cheeses. For special purposes a medium can be prepared from the product involved; butter serum, extracts or suspensions of cheeses, etc., have been employed as bases for agar. Special media adapted to the growth or differen-

tiation of specific organisms often are helpful, such as lactate agar for the propionic acid organisms.

For anaerobic or microaerophilic organisms, the oxygen supply of the plates can be adjusted. In some instances it is desirable to restrain the growth of certain organisms so that species of particular

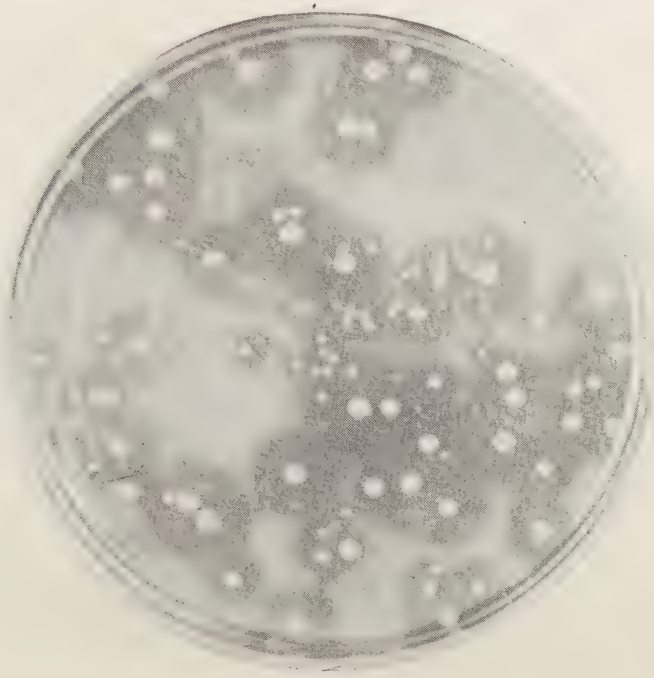


Figure 1. Agar plate poured with added milk. Some of the colonies are proteolytic, as shown by clearing of the casein.

interest can be counted or isolated more readily. This sometimes can be accomplished by addition of an organic acid, sodium chloride, or some comparable material.

Differential Media. Media which permit detection of proteolytic, acid-forming, lipolytic, or other types of organisms are of special interest in investigations on dairy products.

Proteolytic and Acid-forming Organisms. Ayers and Mudge [24] proposed various milk powder agar media that permit detection of proteolytic and acid-forming organisms through their action in digesting and precipitating the casein, respectively. They gave higher counts than nutrient agar. Zoller [25] developed a milk powder agar

on which proteolytic and acid-forming organisms can be detected; sodium citrate is used to prevent excessive sedimentation of lime salts and proteins. Simple addition of 0.3 to 1.0 ml of sterile skim milk to a plate before pouring with various media permits detection of proteolytic organisms and, when sufficient sugar is present, of acid-forming organisms also. If the medium is in relatively large volumes, the required amount of milk can be added to it just before the plates are poured. Safford and Stark [26] used an agar medium containing skim milk and glucose, together with peptone and beef extract, on which acid-producing and proteolytic bacteria were differentiated. With this medium the counts on 618 samples of pasteurized milk were 2 to 4 times as high as on nutrient agar, whereas the counts on 137 samples of raw milk were only slightly higher; the colonies also were larger. The medium supported growth of bacteria responsible for mastitis in cows.

For differentiation of acid-forming and nonacid-forming bacteria, Wade *et al.*[27] used a medium that included, per 100 ml, 0.4% calcium carbonate and 0.2 ml of 1.6% alcoholic solution of bromcresol purple; it was found expedient to add the carbonate from a water suspension by means of a pipette. The carbonate delays diffusion through the agar of the acid produced by acid-forming colonies and, as a result, the indicator changes color only in the zones surrounding the colonies. Without the carbonate, rapid diffusion of acid makes it difficult to distinguish between acid-forming and nonacid-forming colonies.

Lipolytic Organisms. Lipolytic organisms can be detected on plates in various ways [28] if fat is dispersed in the medium at the time of pouring; observations are made best with low magnification. A suitable fat distribution can be obtained with a fat emulsion prepared as follows: Three ml of fat (cottonseed oil, butter fat, or other fat) and 0.5 g of agar are sterilized in 100 ml of water and, after cooling, the fat is dispersed by vigorous agitation. The mixture should be rewarmed before the agitation if butter fat, or other fat that is not liquid at room temperatures, is used. About 0.5 ml of the emulsion is added to each plate just before it is poured with the selected medium.

Fat globules around colonies of actively lipolytic organisms have an opaque appearance that is conspicuous with a low melting point fat, such as cottonseed oil. When butter fat is used it should be melted by holding the plates at 98.6 F (37 C) just before the observations are made. Weakly lipolytic organisms often are difficult to detect with this method.

Partly hydrolyzed fat is differentiated readily by flooding the plates for 30 min with 1 to 1,500 aqueous nile blue sulfate solution and then rinsing with water; if the rinsed plates are held several hours at about 113 F (45 C), the staining is intensified. Globules of unhydrolyzed fat are pink and globules containing partly hydrolyzed fat are blue

Figure 2. Agar plate poured with added fat emulsion. Around most of the colonies the finely divided fat globules are opaque, indicating lipolysis.

so that around colonies of actively lipolytic organisms a zone of blue globules is evident. With weakly lipolytic organisms the blue globules may be found only under the colonies. A saturated solution of copper sulfate also can be used to flood the plates; [29] with this reagent the hydrolyzed fat is stained bluish-green, owing to formation of a copper soap, but the differentiation is not as sharp as with nile blue sulfate. With either reagent, washing of the organisms over the surface of the plate is a serious objection when colonies are to be picked.

A sterile aqueous solution of nile blue sulfate can be added to the medium and fat emulsion at the time of pouring the plates; [28] the usual

concentration is 1 part dye to 10,000 or 15,000 parts of medium. With this procedure there is an unusually good differentiation of partly hydrolyzed and unhydrolyzed fat, but the nile blue sulfate is toxic for various organisms, especially streptococci and micrococci, so that both the total and lipolytic counts are low.

In order to impart sufficient color to the fat droplets without making the medium toxic for organisms, Knaysi [30] suggested shaking the neutral fat to be tested with a saturated aqueous solution of sulfate or chloride of nile blue, in the proportion of 1 ml of the dye solution to 10 ml of fat. The deep red fat is separated from the blue solution with a separatory funnel, washed several times with distilled water, and sterilized in the autoclave; 1 ml of the red fat then can be shaken with 100 ml of the medium and used for plating. The stock of sterile red fat can be stored in the icebox for several weeks.

For the detection of lipolytic bacteria, Starr [31] suggested an agar containing cottonseed oil emulsion and spirit blue as the indicator. Sterile plates of the medium are pale lavender in color. Colonies of lipolytic organisms are recognized by development of a permanent deep-blue color beneath and surrounding them. There was no evidence of inhibition of growth or lipolysis by the indicator.

If tripropionin or tributyrin [32] is dispersed in an agar medium as fine globules, there is a disappearance of the globules around colonies of lipolytic organisms because the products of hydrolysis are completely soluble. However, since these simple triglycerides are hydrolyzed more easily than natural fats, the results may be misleading.

Both the proteolytic and lipolytic organisms can be differentiated on plates poured with agar containing milk and finely divided fat. After incubation the proteolytic colonies are detected on the basis of clearing of the casein around them, whereas the lipolytic colonies are detected on the basis of the opaque condition produced in the fat globules or the plates can be flooded with nile blue sulfate solution in the usual way. Many of the actively lipolytic organisms also are proteolytic.

Oxidase-producing Organisms. Castell and Garrard [33] detected oxidase-producing organisms by flooding plates in which colonies had grown with a 0.5% aqueous solution of either *p*-aminodimethylaniline hydrochloride or tetramethyl-*p*-phenylenediamine hydrochloride. As soon as the agar surface was completely covered with the dye solution, it was poured off and the plates left with their covers removed. Fresh dye solutions were almost colorless. After contact with the

dyes, oxidase-forming colonies very shortly assumed a deep color. The dyes differ in the color produced and in sensitivity.

Incubation Conditions. Incubation at 70 or 86 F (21.1 or 30 C) for several days commonly gives higher counts on dairy products than incubation at 95 or 98.6 F (35 or 37 C) for 48 hr and can be used for special counts since it is not necessary that results be obtained quickly. Among the organisms present in milk and its derivatives, species that fail to grow at 98.6 F often are encountered. For definite groups of organisms, temperatures that are especially favorable can be used.

Thermophiles and Psychrophiles. Thermophilic and psychrophilic bacteria are common in dairy products, and counts of these groups sometimes are desired. With either group, various media can be used. For typical thermophiles the plates are incubated at 131 F (55 C) for 2 days and for typical psychrophiles at 41 F (5 C) for 7 days; [1] these conditions can be varied for special purposes.

Coliform Organisms. The numbers of coliform organisms in a dairy product sometimes permit conclusions that cannot be drawn from total counts. They may indicate contamination of a pasteurized product, objectionable contamination of a raw product, the cause of gas formation in cheese or other dairy product, etc. Coliform estimates also can be used in testing the sanitization of equipment.

The general numbers of coliform organisms in a product or rinse solution can be determined by plating on violet red bile agar, desoxycholate lactose agar, etc.[1] By using various amounts of inoculating material in a series of tubes of brilliant green lactose peptone bile, formate ricinoleate lactose peptone broth, etc.,[1] the most probable number of coliform organisms can be estimated.

Yeast and Mold Counts. Yeasts and molds grow on various media used for bacterial counts and colonies of these organisms, especially yeasts, may be included in bacterial counts. In some cases it is desirable to make plate counts of the yeasts and molds (1) to check the extent of contamination of products that should be free of the organisms, e.g., butter, or (2) to study the numbers under conditions permitting contamination and growth, e.g., cheese surfaces. Growth of bacteria on the plates is prevented by acidifying the melted and cooled medium to a *p*H (usually 3.5) low enough to exclude bacterial activity.

Counts of yeasts and molds have been used most extensively on butter to detect unsatisfactory methods of manufacture, and a standard procedure [1] using potato dextrose agar has been developed (see p. 445). Commonly, the two groups are counted together because of

the difficulty of easily and accurately distinguishing between them, and they are equally indicative of contamination.

The method used for butter, or a modification, also can be applied to such products as cottage cheese, fermented milk, cheese surfaces, mold-ripened cheeses, etc. Smearing the inoculating material on the surface of acidified, solidified agar is advisable when yeasts and molds are to be counted separately since surface colonies simplify the differentiation.

DYE REDUCTION TESTS

The methylene blue and resazurin reduction tests which are described in Chapter 2 are used rather extensively for determining the bacteriological quality of raw milk. The Milk Ordinance and Code [13] of the U. S. Public Health Service recognizes these tests as methods for grading raw milk. The reduction tests do not indicate the actual number of bacteria in milk but measure a type of bacterial activity which can be used to classify milk as *acceptable* or *nonacceptable*. Since bacterial counts and reduction tests represent two entirely different methods of assessing milk quality, the results of one method should not be interpreted in terms of the other. Certain samples of milk show a definite relationship between bacterial estimates made by the plate or direct microscopic count and reduction times; other samples show no relationship. A sample of milk tested for bacteriological quality by two or more methods may be placed in a different grade by each method. The method which places the sample in the highest grade probably has failed to detect certain bacteria that are detected by the other methods.

Only certain samples of high-count milk are classified as nonacceptable by the reduction tests. The tests are perhaps less useful in detecting milk with a high count due to utensil contamination than in detecting milk with a high count due to improper cooling.

ADDITIONAL PROCEDURES FOR COUNTING BACTERIA

Although most bacterial counts on dairy products have been made either with the microscopic or plate method, additional procedures are useful under some conditions.

Burri Smear Culture Technic. Burri [34] suggested a method of making counts of the organisms in milk by smearing it on the surface of an agar slope with a standardized loop and counting the colonies that

develop on incubation. His general observations on the procedure are: A loop adjusted to deliver 1 cu mm, as determined by weighing the milk, is very satisfactory. However, any ordinary loop can be used; it is calibrated by determining the number of bacteria in milk with the plate method and comparing this with the number of colonies developing when a loop of the milk is used to inoculate a plate. Relationship of the volume of milk delivered by the loop to 1 ml should be verified by repeated trials. Since as many as 100 colonies can be counted on a slope with a magnifying lens, a 1-cu-mm loop of undiluted milk gives counts from 1,000 to 100,000 per ml. By diluting the milk 1 to 100, counts from 100,000 to 10,000,000 can be obtained; counts made with water dilutions should be multiplied by 1.4 because of the change in surface tension that influences the amount of material delivered. Various media and incubation conditions can be used with the method. The agar slopes should be dry since water of condensation in the tubes is objectionable. With low-count milk, more than one loop can be used per slope. The procedure is simple, economical, tends to avoid air contamination, and permits good colony differentiation since all the colonies are on the surface. The slopes can be kept for extended periods if additional observations are desired. The method gives results that are somewhat higher than those obtained with the plate method.

Cunningham and Andrews [35] found that the percentage error with the Burri method was markedly higher than with the plate method. They consider the inaccuracy of the Burri method is due partly to errors in loop measurements and partly to inferiority of conditions for growth on slopes.

Oval Tube Technic. Myers and Pence [36] suggested a loop measurement, oval tube technic for making laboratory pasteurization tests on individual producer samples after the technic was found to give reliable counts on heated milk. A 5-ml sample of milk is laboratory pasteurized and then vigorously shaken 50 times to compensate for the breaking up of clumps in the usual plating. A loopful (0.001 or 0.01 ml) of the pasteurized milk is transferred to an oval culture tube containing about 4 ml of agar that has been sterilized and cooled to 113 F (45 C). The loop is moved back and forth through the agar to remove all the milk. The mixture of agar and milk then is shaken by swinging the tube back and forth through a small arc for about 5 sec. The tube is placed on a table with the stoppered end resting on a strip of wood or metal ⅛ in. thick to permit the agar to flow to a point 2.5 to 3 in. from the bottom of the tube. When the agar has

solidified, the tube is placed in a special wire rack in a horizontal position but inverted. After incubation, the colonies are counted over a well-lighted colony counter. Myers and Pence prefer oval tubes without an indent, since they are easy to inoculate and clean. A tube can be plugged readily by wrapping the cotton around a strip of folded aluminum foil which then is folded in the middle and pushed into the tube.

The oval tube technic can be applied to the examination of dairy products in a variety of ways, particularly when the approximate bacterial count of the material is known so that suitable numbers of colonies can be obtained without preparing an excessive number of tubes; medium and incubation conditions can be varied as desired. Compared to the usual plating procedure, the method saves time, materials, incubator space, etc. It also can be applied to the counting of yeasts and molds in dairy products.

Roll Tube Technic. The original roll tube technic involved introducing the test material into gelatin in an ordinary test tube, closing the tube with a cotton stopper covered by a rubber cap, and rotating it by hand under a stream of cold water until the gelatin congealed on the walls. The procedure has been refined by introducing motor-driven rotating devices, special tubes, agar media, etc. It possesses the usual advantages of any procedure in which tubes are used instead of plates.

Prouty *et al.*[37] found that the roll tube method gave slightly lower counts than the plate method, but the difference did not justify its rejection. The tubes were the more difficult to count. Thomas *et al.*[38] reported that, on the whole, counts by the roll tube technic tended to be higher than plate counts; they suggested that rolling the tubes results in a more thorough mixing of the inoculum and medium which may break up more bacterial clumps.

Microscopic Colony Count. Frost [39] devised a microscopic colony count, or little plate method, for counting bacteria in milk. The general procedure is as follows: A small amount of milk, commonly 0.05 ml, is mixed with liquefied agar at about 113 F (45 C) on a sterile glass slide and the mixture spread over a definite area, such as 4 sq cm. After hardening, the preparation is held in a moist chamber for some hours to permit the organisms to develop colonies, and then quickly dried. Finally, the slide is stained with methylene blue or thionine, the colonies in a number of fields counted under a microscope, and the organisms per milliliter of the milk then calculated; the magnification

used can vary widely. Milk that is expected to give a high count is diluted before the little plates are prepared.

The advantages of the method as listed by Frost [10] are:

1. It is rapid, requiring not more than 7 or 8 hr.
2. It is reliable in that failure due to spreaders or improper dilution is unlikely.
3. A relatively large amount of milk is studied, usually 0.05 ml.
4. A permanent record is provided.
5. It requires only a small amount of glassware and medium.
6. The little plates are quickly made.
7. It is simpler than the plate method.

Hatfield and Park [41] reported favorably on the little plate method. With it, certified milk showed the smallest average variation from standard plate counts. The larger variations with pasteurized milk and milk to be pasteurized were about equally divided between counts higher and counts lower than the plate results. Bryan *et al.*[42] found that incubation of Frost little plates in a moist chamber at 98.6 F (37 C) for 4 hr yielded bacterial colony counts on milk and cream comparable to standard plate counts.

Field Procedures. When it is necessary to transport the equipment for making counts, bottles with cotton stoppers or screw caps can be used instead of plates; a type of bottle with at least one flat side on which it can be rested to give a layer of agar of uniform thickness is advisable. The containers are sterilized with agar in them and, when needed, the agar is melted in boiling water and cooled for inoculation. The inoculum is added, usually with a loop although pipettes and water blanks may be employed, and then the agar is solidified. After incubation, the colonies are counted.

Bell [43] devised a special rectangular culture flask of heat-resistant glass for use in milk receiving stations, etc. It has an offset, round neck so that 10 ml of sterile agar and 0.001 ml of milk can be mixed without wetting the cotton stopper.

COLLECTION AND CARE OF SAMPLES

Samples of dairy products that are to be examined bacteriologically must be collected, cooled, and held under conditions that avoid contamination and prevent growth of the organisms present. Standard procedures for the collection and handling of routine samples of the usual types have been developed.[1]

Collection of samples for special counts, or for control of plant

processes, sometimes presents difficulties. Lines for carrying milk or other materials can be tapped and outlets attached, but when samples are obtained in this way it is essential that the outlets be steamed and well flushed with the product before the sample is collected.

In sampling large volumes of milk, cream, etc., in tanks, thorough agitation is important, particularly when the material is made up of small lots from many sources and there is no agitation during the collection. If the tank has no sampling valve and the level of the product in it is beyond the reach of sampling tubes, etc., the product can be sampled by lowering a sterile bottle into the tank with precautions against contamination.

Proper collection and holding of samples are essential in all bacteriological work. Undoubtedly, much effort has been expended on samples that could not give reliable results because the collection and/or care was faulty.

LINE TESTS

Line tests, in which a series of samples is taken during a processing operation, are used in connection with bacteriological control of dairy products and are very useful in locating irregularities in plant equipment and/or in its operation. It is essential that the samples be collected and held under proper conditions; otherwise the results may be misleading.

If milk supplied by a certain plant has a high bacterial count, a line test can be run in an attempt to determine the cause. The usual procedure is to take a series of samples, beginning with the raw milk, at various points in the processing; the points often are determined by the opportunities for collection of special samples. If an undesirable microbiological condition is found in the product of a butter plant, a series of samples can be taken, beginning with the raw cream and including freshly pasteurized cream in the storage vat, cream after holding, cream in the churn, cream after the churn has been operated briefly, buttermilk and granules as soon as granules have formed, wash water, washed butter, worked butter, butter as it comes from the churn, and butter after various holding periods. Results on a series of samples taken during the processing of milk or the manufacture of butter or other dairy product often show the source of excessive numbers or objectionable types of organisms so that correction is simplified.

Line tests also are useful in determining changes in the numbers and types of organisms during the normal production of a dairy prod-

uct, such as milk powder, various cheeses, etc. Information on a normal operation is helpful when difficulties develop and the cause must be determined.

REFERENCES

1. *Standard Methods for the Examination of Dairy Products* (last ed.). American Public Health Association, Inc., New York, N. Y.
2. *U. S. Public Health Repts.*, **58**, 1641 and 1605 (1943).
3. *U. S. Public Health Repts.*, **65**, 931 (1950).
4. *J. Milk and Food Technol.*, **14**, 105 (1951).
5. *J. Dairy Sci.*, **3**, 494 (1920).
6. *J. Milk and Food Technol.*, **10**, 269 (1947).
7. *Butler Univ. Botan. Studies*, **8**, 139 (1948).
8. *N. Y. State Assoc. Dairy & Milk Inspectors, 15th Ann. Rept.*, 229 (1941).
9. *U. S. Public Health Repts.*, **30**, 2349 (1915).
10. *Am. J. Public Health*, **8**, 913 (1918).
11. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 75 (1920).
12. *Intern. Assoc. Dairy and Milk Inspectors, 22nd Ann. Rept.*, 225 (1933).
13. *Milk Ordinance and Code*, 1953 Revision of Public Health Bull. 220.
14. *J. Milk and Food Technol.*, **11**, 5 (1948).
15. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 86 (1921).
16. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 74 (1919).
17. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 98 (1923).
18. *J. Milk Technol.*, **5**, 67 (1942).
19. *Am. Milk Rev.*, **9** (10), 24 (1947).
20. *Am. J. Public Health*, **33**, 401 (1943).
21. *J. Milk Technol.*, **6**, 231 (1943).
22. *J. Dairy Research*, **2**, 81 (1930).
23. *J. Bacteriol.*, **1**, 481 (1916).
24. *J. Bacteriol.*, **5**, 565 (1920).
25. *Am. J. Public Health*, **13**, 384 (1923).
26. *J. Dairy Sci.*, **18**, 539 (1935).
27. *J. Bacteriol.*, **51**, 787 (1946).
28. *Ia. State Coll. J. Sci.*, **11**, 343 (1937).
29. *J. Bacteriol.*, **25**, 433 (1933).
30. *J. Bacteriol.*, **42**, 587 (1941).
31. *Science*, **93**, 333 (1941).
32. *J. Bacteriol.*, **27**, 69 (1934).
33. *Food Research*, **5**, 215 (1940).
34. *Proc. World's Dairy Congr.*, 690 (1928).
35. *J. Dairy Research*, **5**, 29 (1933).
36. *J. Milk Technol.*, **4**, 18 (1941).
37. *J. Milk Technol.*, **7**, 5 (1944).
38. *Proc. Soc. Agr. Bacteriologists*, 41 (1940).
39. *J. Infectious Diseases*, **28**, 176 (1921).
40. *J. Bacteriol.*, **2**, 567 (1917).
41. *Am. J. Public Health*, **12**, 478 (1922).
42. *J. Dairy Sci.*, **25**, 827 (1942).
43. *Food Inds.*, **16**, 797 (1944).

Tests for the General Quality of Raw Milk

2

In addition to enumeration of the bacteria in raw milk, various other tests are used to determine the general quality. These involve (1) detection of the products of growth of organisms; (2) rate at which changes are produced by the contained organisms; (3) presence of certain types of organisms, including types objectionable in milk processing; and (4) tests intended to detect udder infections. Often the tests are simple so that they can be carried out by persons without special training and can be applied quickly to many samples. Certain of the tests are applicable to the examination of cream.

TESTS FOR PRODUCTS OF GROWTH OF ORGANISMS

Under the usual conditions of producing and holding raw milk, extended growth of organisms results in the formation of products that give an abnormal odor and flavor and, eventually, a definite increase in acid and also an instability that sometimes is detected on mixing with alcohol under standard conditions.

Examination for Odor and Flavor

Milk received at dairy plants commonly is examined for odor and sometimes for flavor. Plant employees may hesitate to taste the raw product because of the possibility of contracting a milk-borne disease. Many flavor defects are recognized readily by the odor alone; perhaps the outstanding exceptions are bitterness and saltiness and, ordinarily,

these are of relatively little importance in milk. The odor when the cover of a can of milk is first raised is especially valuable in indicating a slight defect.

Abnormal odors in milk may be due to any one of a number of causes, such as absorption of odors, materials consumed by the cows, condition of the cows, chemical action, addition of foreign materials, and action of micro-organisms. Those due to organisms (see p. 103) are of various types, but an odor suggestive of acid, often modified by some other odor, is the most common. The experienced grader of milk and cream learns to recognize the causes of abnormal odors and, with those due to organisms, often can identify the particular species or group involved. Recognizing the cause of a defect is especially helpful in advising the producer as to methods of correction.

Abnormal Flavors Not Due to Organisms. Various factors other than activity of organisms may be responsible for abnormal flavors in milk and cream. The following grouping includes most of them.

1. Absorption of Odors. Odors are absorbed quickly from the surroundings by exposed milk and cream. Absorption is a common cause of such flavors as gasoline, fly-spray, disinfectant, etc., and may play a part with others, such as feedy and barny flavors. At one time it was believed that milk would not absorb odors when it was warmer than the surrounding atmosphere, but it now is generally recognized that absorption occurs whether the milk is warm or cold. The rapidity with which milk can absorb odors is shown by the investigations of Russell; [1] horse manure was absorbed in 0.25 hr, cow urine in 0.5 hr, and silage in 1 hr. The fat is the constituent mainly involved in flavor absorption. Trout and McMillan [2] found that raw or pasteurized milk seemed to absorb odors slightly more than homogenized-heated milk, wherein creaming was inhibited. Absorption was slightly greater when the fat was in the liquid state but also occurred when the fat was in the solid state. Absorption appeared to be primarily a surface problem, the absorbed odor being confined largely to the upper 10% of a 10-gal can. When this layer was thoroughly mixed with the under layers, the odor of the substance to which the milk was exposed could not be identified.

2. Materials Consumed by the Cows. Materials consumed by producing animals may play an important part in determining the flavor of milk and cream. Feed flavors are imparted to the milk through the bodies of the cows and many feeds, such as silage, alfalfa, sweet clover, turnips, garlic, various weeds, etc., are responsible for

definite abnormal conditions. Drugs and drinking water having pronounced flavors may act in the same way.[3]

After a feed is consumed by a cow, a certain period is required before the flavor is evident in the milk. The flavor then increases in intensity for a time, later decreases, and finally disappears. Roadhouse and Henderson [4] noted that, when the juice expressed from 25 lb of green alfalfa was administered as a drench to each of 4 cows, feed flavor was detected in the milk in 20 min. The flavor was most pronounced in the milk drawn 45 min after administering the juice and was less noticeable in the milk drawn after 2 hr. Babcock [5] reported detection of a garlic flavor in milk when the samples were taken 1 min after feeding garlic; intensity of the flavor increased as the interval between the feeding and milking increased, until after 10 min it was very pronounced. Strong garlic flavor was found in milk drawn 2 min after the animals had inhaled the odor of garlic for 10 min. Garlic odor was evident in samples of blood drawn after feeding garlic.

Certain feed flavors are largely seasonal. Garlic flavor is most common in the spring because garlic is one of the first plants to start growth and the animals then eat it readily; but it may occur at other times. Certain weed flavors are encountered more frequently in the late summer when pastures are dry and the cows eat plants that were rejected earlier. Feed flavors often are more pronounced in cream than in the milk from which it came, owing to the absorbing power of the fat. Effect of feed on the flavor of milk sometimes is evident when the cows are kept on one type of pasture during the day and another during the night. Flavors in milk due to the materials consumed by the producing animals represent instances of absorption before the milk leaves the udder. The constituents responsible for the flavors probably are carried to the udder through the blood and lymph.

3. Condition of the Cows. When a cow is advanced in lactation, the milk frequently shows an undesirable flavor that commonly is described as rancid, bitter, or salty, and an abnormal flavor may be present in milk from infected udders. With both these conditions there is great variation among different animals in the extent of the undesirable flavor, and it even may be entirely lacking.

The flavor present in milk as a result of advanced lactation often is not noticed until the milk has stood for a time. After it is evident, it usually increases with the age of the milk. Eckles and Shaw [6] reported the flavor of the milk of 4 animals advanced in lactation as rancid and bitter combined and stated that it developed within 24 hr.

Palmer [7] found this abnormal flavor due to the activity of lipase which hydrolyzes the fat with liberation of fatty acids; the lower volatile fatty acids, especially butyric, largely cause the objectionable flavor. The enzyme is active, even at fairly low temperatures, but can be destroyed by pasteurization. Various factors influence its activity and these have been studied extensively (see p. 296) because of the importance of the enzyme in various dairy plant operations. In advanced lactation there often is a high chloride content that may, in part, be responsible for the abnormal flavor since this frequently is described as salty. The off flavor in the milk of animals with infected udders presumably is due to variations in the composition which result from the condition of the udder tissues.

Individuality of the animal also may be of importance from the standpoint of the flavor of the milk, entirely aside from such factors as feed consumed, stage of lactation, etc. Certain cows, even under very favorable conditions, give milk with more or less of an unpleasant flavor, and this may continue through succeeding lactation periods. There may be differences in the flavor of the milk from the various quarters and also from various portions of the milking. The foremilk sometimes has a rather pronounced unpleasant flavor and the strippings may have either a less or more pleasant flavor than the main portion of the milking, depending undoubtedly on whether the off flavor is one associated with the fat.

Flavors due to the condition of a producing animal are noted less often in the milk from herds than in the milk from individual cows since with herd milk an off flavor in the milk of any one animal is more or less obscured by the mixing of the milk. However, these flavors sometimes involve considerable volumes of milk and cream.

4. Chemical Action. When milk or cream is exposed to sunlight, it rather quickly acquires an off flavor which, when well developed, suggests tallow and often is referred to as tallowy. The condition is encountered most often in bottled milk left on the porch for some time after delivery. Hammer and Cordes [8] noted an off flavor in milk exposed to sunlight for only 10 min, and definite tallowiness was observed with exposures as short as 45 min. Exposed milk had a chalky appearance and when churned yielded a lighter colored fat than the unexposed milk. Tallowiness develops on exposure to sunlight at very low temperatures and has been noted in ice cream held at such a low temperature that it remained firm. Frazier [9] reported an off flavor in whole milk which had been exposed to diffuse daylight for 8, or more, hours at freezing temperature. The light apparently acted as a catalyst

in the oxidation of milk fat. The defect developed more rapidly in pasteurized than in raw milk.

A flavor that is referred to as oxidized sometimes is present in milk held with minimum exposure to light. It appears most often in pasteurized milk at certain seasons but also is encountered in raw milk. Development of the flavor is influenced by various factors such as presence of dissolved metal, particularly copper, feed of the animal, individuality of the animal, etc. The flavor appears to be due to an oxidation of one or more constituents of the milk and probably is related to the off flavor caused by light.

5. Addition of Foreign Materials. Foreign materials occasionally are added to milk and the flavor influenced in this way. Soap may be left in a utensil; a pail or can may be used in transporting oil, kerosene, or gasoline and then be used for milk; or material on the hands of the milker or the udder may be washed into the milk. Ointments or salves containing phenol, when applied to the teats, may contaminate milk and cause serious off flavors. Phenol reacts with the chlorine of bactericides to form chlorophenol, a compound which affects the flavor of milk even when present in extremely small amounts. In some instances off flavors have resulted from foreign materials (bactericides, acid neutralizers, etc.) added to milk in an attempt to improve the bacteriological quality and/or flavor.

Titration for Increased Acidity

Milk sometimes is titrated for the purpose of detecting increases in acid. Because the initial acidities of different lots of milk vary so widely, such tests are only very crude; they are much less sensitive than organoleptic examination by an experienced person who commonly detects very small changes in acid. Definite increases in titratable acid due to growth of bacteria occur only when large numbers are present. The chief value of titrations is in connection with establishment of standards that exclude from certain uses milk with relatively high acidities.

Variations in the initial acidities of lots of milk depend in part on the composition. Some lots of milk with high total solids contents may have acidities higher than any standard likely to be established, even when there has been no opportunity for growth of organisms, whereas other lots may have such low total solids contents that even with considerable increases in acid the acidities are below any such standard. Mixing of milk from a number of animals eliminates some of the variations in initial acidities, but they still are evident in herd milk, espe-

cially when different breeds are involved. The acidity of fresh cream is lower than that of the milk from which it came, the extent being related to the fat content, but often the same standards are applied to the two products.

Titrations have been of special value in the grading of cream for butter. The butter industry normally uses some cream in which rather extensive growth of organisms has occurred. In such cream most odors and flavors due to organisms are objectionable and, commonly, they are not as conspicuous with an acidity of 0.2%, or less, as with a higher one. Cream which has developed considerable acid may be placed in more than one grade. Grading of cream on the basis of acid can be carried out rapidly by mixing cream and standard alkali, containing indicator, in definite proportions and then noting whether the reaction is acid or alkaline. By varying the strength of the standard alkali, acidities above any desired point can be detected. The same procedure can be used with milk.

Acidity of Fresh Milk. Freshly drawn milk shows considerable variation in acidity, calculated as lactic acid, and in *p*H. In 86 tests on individual samples from 31 cows, Sommer and Hart [10] noted acidities from 0.102 to 0.257%; 45 of them were above 0.18%. The *p*H values of more than 300 samples of fresh milk, each representing only a portion of the milking, were found by Van Slyke and Baker [11] to range from 6.50 to 7.20, with 80% of the samples under 6.76. In general, with a decrease in acidity there was a marked tendency toward a decrease in specific gravity and in percentages of fat, total solids, solids-not-fat, casein, and lactose but an increase in percentages of proteins other than casein and in ash and chlorine.

McInerney [12] reported acidities from 0.10 to 0.22% with fresh milk from individual cows in two herds. With the milk of a number of cows, Rice and Markley [13] noted variations in acidities from 0.086 to 0.229% and in *p*H values from 6.432 to 6.898. In general, high-acid milk contained more nutrients and was particularly high in phosphates. The investigators concluded that carbon dioxide is responsible for 0.01 to 0.02% acidity in milk, casein for 0.05 to 0.08%, albumin for $<0.01\%$, and phosphates for the remainder, although citrates might account for 0.01%. Robinson and Samson [14] concluded that inorganic phosphates are the primary cause of acidity of fresh milk.

Houston [15] studied >2,500 samples of milk from 39 complete and 38 incomplete lactation periods. The average acidity for all the tests was 0.189% and for the tests involving complete lactation it was 0.190. Average acidities for individual lactations ranged from 0.154 to 0.235%,

whereas acidities of individual milkings ranged from 0.105 to 0.270. In general, the acidities were highest at the beginning of the lactation period and lowest at the end. The fall was not uniform but was fairly rapid during the first 8 weeks of lactation and also during the last 7 weeks.

Sommer [16] found the *p*H values on 386 samples of milk ranged from 6.22 to 6.77; both milk and udders were carefully examined to detect subclinical mastitis and the definite and doubtful samples eliminated.

Caulfield and Riddell [17] found the acidities of 811 samples from individual cows of various breeds ranged from 0.098 to 0.295%. Average percentages for the various breeds were Ayrshire 0.160, Holstein 0.161, Guernsey 0.172, and Jersey 0.179, and the average for all breeds was 0.166. The acidity of colostrum was high, averaging 0.44% for the first milkings from a group of 10 cows. At the third milking the acidity averaged 0.26%, at the sixth milking 0.21%, and there was a gradual decline during the first 15 to 20 days of lactation. With a group of 36 cows, the acidities showed a gradual decline throughout the lactation period; the most pronounced changes were at the beginning and end of lactation, and from the second to the seventh month of lactation the acidities remained fairly constant.

There often is some fluctuation in acidity throughout a milking, the foremilk being comparatively low, the middle milk highest, and the strippings between the two. Influence of feed on the acidity of milk is apparently negligible; Turner and Beach [18] noted no effect from silage, and Sommer and Hart [19] added up to 120 ml of concentrated sulfuric acid to the ration of a normal cow without affecting the acidity of the milk. Duncombe [20] found that feeding lactic, butyric, acetic, or phosphoric acid to cows had no appreciable effect on titratable acidity or *p*H of the milk, whereas when the cows were changed from a dry to a pasture ration there was a decrease in titratable acidity but practically no change in *p*H.

Alcohol Test

The alcohol test consists of mixing equal parts of milk and ethyl alcohol of a definite percentage in a tube and noting whether coagulation occurs. With normal milk the mixture breaks clear from the wall of the tube; with milk showing certain abnormalities the wall is clouded with fine particles of curd and the mixture shows a precipitate of milk constituents. The alcohol employed usually has varied from 68 to 75% by volume. It can be prepared by diluting high-grade alcohol with distilled water. The diluted alcohol should be tested

for acid since acetic acid sometimes is present and may influence the results. The test usually is made at room temperature, using 2 ml of milk.

Conditions Causing Coagulation. From the data available it is evident that products of the growth of organisms may be responsible for coagulation in the alcohol test, and also that certain other factors may be involved.

One of the causes of a positive test is the presence of developed acid in milk. However, Dahlberg and Garner [21] found no direct relationship between coagulation of milk with alcohol and its acidity, although milk high in acid gave coagulation in the majority of cases. Development of a bacterial rennin in milk may be a factor in the test; Sommer and Binney [22] noted that the coagulating enzyme produced by 856,000 rennin-forming organisms per ml was sufficient to give a positive result. Ayers and Johnson [23] found no definite relationship between numbers of bacteria in milk and coagulation with alcohol.

Colostrum and milk from cows advanced in lactation usually are positive with the alcohol test and some milk from abnormal udders also is positive. This suggests that certain abnormalities in the composition of milk result in coagulation when the milk is mixed with alcohol. If milk that coagulates with alcohol is mixed with normal milk, the mixture may not coagulate.[23]

Sommer and Binney [22] investigated the influence of the salt balance of milk on the alcohol test and noted that a slight increase in magnesium or calcium gave a positive result, whereas an increase in potassium, sodium, chloride, citrate, or phosphate did not. The effect of magnesium and calcium is counteracted by citrates and phosphates so that a positive test depends mainly on the relative amounts of the four constituents. It was noted that a high calcium content in a ration fed to cows may cause a positive alcohol test on the milk. Weimar [24] reported instances in which a positive alcohol test was due to feeding silage of poor quality. Experimental feeding with three animals showed that the effect of alcohol on the milk could be changed by varying the feed.

Modifications of the Test. Chalmers [25] modified the alcohol test by subjecting the milk to a preliminary incubation of at least 9 hr at 98.6 F and then adding 83% (by volume) neutral alcohol containing 0.02% methyl red. The incubation permits development of the contained organisms under standard conditions before the milk is examined for products of their growth, while the indicator gives information as to

the acid present. Cooling the milk before the reagent is added appears to be important from the standpoint of the color obtained.

To facilitate the detection of alcohol precipitation, and to obtain an indication of the acidity of the sample, Hughes and Ellison [26] added bromcresol purple to the alcohol. To 500 ml of 95% alcohol, 0.05 g of indicator was added. The alcohol was neutralized with $N/9$ sodium hydroxide to a grayish purple color and water added to obtain the desired density. Assenowa [27] described a micro alcohol platform test in which 1 drop of milk is transferred to a watch glass with a glass rod and 1 to 2 drops of 68% alcohol added. The milk and alcohol are mixed with the convex side of another watch glass by pressing together lightly. The degree of flocculation is observed easily.

Uses of the Test. The alcohol test has been suggested for use with milk intended for condensing or drying. Its detection of unusual salt balances in addition to certain other abnormalities may be of importance in this connection.

Anderson and Wilson [28] used the alcohol test to measure the keeping quality of raw milk. Of 2,588 samples tested, the alcohol test predicted correctly the keeping quality of 86%. Feagan [29] found that tasting plus use of the alcohol test was more reliable for predicting keeping quality of pasteurized milk than reduction tests or plate counts of total or coliform bacteria; the comparison involved 650 samples. Hughes and Ellison [30] noted that the alcohol test did not detect a high proportion of samples which reduced resazurin rapidly.

TESTS INVOLVING RATE AT WHICH CHANGES ARE PRODUCED BY THE CONTAINED ORGANISMS

The rate at which a specific change is produced by the organisms in milk is the basis for a number of tests used to arrive at its microbiological quality. Normally, such a change is related roughly to the numbers of organisms present, but it also is greatly influenced by the species of organisms.

Keeping-quality Test

A simple keeping-quality test can be made on milk by holding samples under more or less definite conditions and, from time to time, observing them for changes. The portions can be held in test tubes or small bottles that have been sterilized or thoroughly steamed and should be transferred under conditions that prevent contamination. A constant temperature is preferable for holding but is not necessary,

and samples in a series can be compared at any temperature reasonably satisfactory for bacterial growth. The period required for coagulation is a convenient basis for comparison although the acidity at which coagulation of milk occurs varies somewhat with different lots; it is a more satisfactory basis than the period required for development of an acid flavor because of the difficulty of deciding when an acid flavor is present.

The character of the curd formed gives information of importance concerning organisms in milk. Gas-producing species, types decomposing casein rapidly, and types producing objectionable odors often can be detected by examination of the curdled material. Such information is of special significance with milk to be made into certain cheeses.

Methylene Blue Test

The methylene blue test involves the determination of the time required for disappearance of the color when a specified methylene blue solution is added to milk under controlled conditions. It is an indirect method of determining the general bacteriological quality of raw milk. Because of its simplicity and the small amount of equipment needed, it is used regularly by many plants for grading milk. The test measures the time required for bacteria to utilize the oxygen in milk and thereby decolorize the dye. In general, the more rapidly the dye is decolorized, the higher the numbers of bacteria; however, there are various exceptions. By examining milk samples for dye decolorization at different intervals, they can be grouped rapidly into classes or grades.

A standard procedure should be followed in making the test so that irregularities in results are kept to a minimum.[31] In 1935, Thornton and Sandin [32] recommended that methylene blue thiocyanate be substituted for the chloride because of reproducibility of the former dye.

Variations in Reducing Powers of Different Organisms. The failure of the methylene blue test to correlate with plate counts on certain samples of milk is due to variations in reducing powers of different organisms. In general, *Streptococcus lactis* [33] and the coliform bacteria [34,35] reduce the dye rapidly, whereas certain of the more heat resistant bacteria reduce it slowly, if at all. Davis and Lines [36] reported an associative action by bacteria in the reduction of methylene blue which usually overshadowed individual variations in reducing power.

Effect of Storage of Samples on Reduction Time. Some plants receive milk rather late in the day and find it inconvenient to make tests at the time the milk is received. Even with milk received early in the day, the first samples collected may be held several hours before being tested. The standard procedure permits storage of milk at 32 to 40 F for a period not to exceed 24 hr before testing.[31] The effect of storage on dye reduction has varied in different studies. Some investigators [37,38,39] have reported that the reduction time is increased by low-temperature storage of samples, while others [40,41] have noted no significant change in reduction time.

Effect of Inversion of Test Portions. Early observations indicated that occasional inversion of tubes of milk containing methylene blue shortened the reduction period because it tended to keep the bacteria distributed through the milk instead of permitting their concentration in the cream layer. Thornton [42] noted that without inversion replicate samples of high-quality milk frequently exhibited serious variations in reduction periods and that the variations tended to increase in frequency and magnitude as the reduction period increased; with inversion of the tubes during incubation the variations practically disappeared and the reduction periods were shortened. Frayer [43] reported that inversion of the tubes resulted in shortening the reduction periods and rendered the tests more significant. With periodic inversion of the tubes during incubation, Abele [44] found a higher, although still imperfect, agreement between plate counts and reduction periods and a nearly complete agreement with respect to plate counts of >200,000 per ml and reduction periods shorter than 6 hr.

Influence of Other Factors on Test. Various other factors also influence the methylene blue test. Most of the studies along this line were carried out before inversion of tubes was the standard procedure, but they probably apply to the test with or without inversion.

Hastings *et al.*[45] found that the reduction period was prolonged as concentration of dye was increased. Age of the methylene blue solution had little if any effect on the reduction period, solutions 2 yr old giving essentially the same results as fresh solutions. In an investigation of the influence of temperature, 82.4, 91.4, and 100.4 F were used; with the majority of samples the highest temperature gave the most rapid reduction, but with certain of them the reduction period at 82.4 F was shorter than at 100.4 F. The bacteria common in milk show great variations in their optimum temperatures and, since certain of these are considerably below 100.4 F, some samples would be expected to reduce more rapidly at lower temperatures.

Fay [46] studied the normal variation in the methylene blue test, making 75 to 100 determinations on each of 19 milk samples, and found that the maximum and minimum reduction periods for each sample were relatively close together. He also noted that various modifications intended to simplify the test did not seriously impair its accuracy. In trials involving variations in the quantity of dye, reduction time was directly related to the amount of dye used.

Light, either from electric lamps or sunlight, hastens the reduction of methylene blue in milk.[38, 47, 48] Various observers suggest that the reduction of methylene blue by light is a reaction distinct from the reduction induced by bacteria. Because of the light effect, the test commonly is carried out in the dark.

Jackson [48] reported that milk as it exists in the udder, or milk drawn anaerobically, reduces methylene blue almost instantaneously, whereas the same milk exposed to oxygen usually takes >10 hr to reduce. The oxidation-reduction potential of anaerobically drawn milk is much lower than that of the same milk exposed to oxygen.

Nilsson [49] noted that methylene blue was reduced rapidly in some individual samples of milk. Such samples contained no formaldehyde, the oxygen content was not depleted, and the bacterial count was low. However, reduction of the dye was accompanied by a rapid drop in the oxidation-reduction potential. In samples of aseptically-drawn milk which reacted normally, there was no decrease in potential as long as there was no growth of bacteria. The reducing system in milk was associated with the fat and was destroyed by heating at 185 F for 30 min.

Several investigators [50, 51] have shown that leucocytes, even when present in considerable numbers, only partially, or temporarily, reduce methylene blue. The slight reduction sometimes noted was nullified by inversion of the tubes which caused the color to return.

Mechanism of Reduction. At one time it was believed that decolorization of methylene blue occurs in two steps: (1) Removal of dissolved oxygen by growing bacteria, and (2) reduction of the dye by the natural reducing system in the milk. Since there is no oxygen in methylene blue, reduction involves the transfer of hydrogen. Thornton and Hastings [52] suggested that it is probable many compounds can act as hydrogen donors. Later, it appeared that the reducing substances formed by the bacteria also are a factor in the reduction and that with some organisms the reducing system formed by bacteria is more important than the reducing system of the milk. Hobbs [34] reported that the most important factor in the reduction is the forma-

ion of reducing substances by the organisms themselves and that the oxygen absorption is incidental rather than essential; on this basis the methylene blue test is an index of bacterial metabolism in the milk.

Visual reduction of methylene blue occurs over a narrow oxidation-reduction potential range (+0.12 to +0.03 v).[53] Among hundreds of milk samples studied by Frayer,[54] no instances were noted in which methylene blue was completely reduced in the presence of more than a minimum of oxygen.

By the use of a dropping mercury electrode, Hoff[55] studied oxygen consumption in milk and its relation to methylene blue reduction. In pasteurized milk inoculated with lactic culture and held at 100.4 F, oxygen consumption increased until the oxygen content reached a very low value. The increasing rate of oxygen consumption was considered due to an increasing number of bacteria. When the bacterial population of milk was held relatively constant by addition of 2,4-dinitrophenol, oxygen consumption per unit of time was directly proportional to the number of bacteria present and was not influenced by the oxygen concentration. Decolorization of methylene blue took place simultaneously with a drop in the oxidation-reduction potential; the drop occurred only when a negligible quantity of oxygen was left.

Numbers of Bacteria Present at Time of Reduction. The numbers of organisms present in milk at the time of reduction of methylene blue are variable. With 38 samples Thornton and Hastings[56] found plate counts from 3.5 to 45 million per ml, with an average of 22 million. Wilson[39] noted plate counts from 3.5 to 70 million and an average of 16.9 million with raw milk, whereas the counts ranged from 7 to 204 million and averaged 103.2 million with pasteurized milk. With milk having a low plate count originally, Davis and Lines[36] found no direct relationship between counts before and after reduction. As the reduction period increased, there was an increase in count after reduction.

Value of Test. When methylene blue is reduced rapidly in a sample of milk, the product generally is of poor bacteriological quality. Samples having a relatively long reduction time may be low in bacteria or they may contain many bacteria of a type that reduces the dye slowly. When milk is cooled properly, so that growth of *S. lactis* and coliform types is restricted, there may be little correlation between the plate count and methylene blue test. Smythe[57] analyzed the results obtained by the methylene blue test on 95,000 milk samples in Australia and noted that the proportion of samples reducing the dye in <4 hr followed the mean atmospheric temperature rather closely.

He concluded that the test was affected more by the holding temperature of milk than by the conditions of production.

The results of Black,[58] presented in the accompanying table, indicate that the general relationship between reduction time and plate count holds in a sufficient number of samples so that results of the test can be used to select farms whose methods of producing and holding milk need improvement.

Percentage of Raw Milk Samples Falling into Various Plate Count Groups When Classified by Methylene Blue Reduction

Reduction Time (hr)	Number of Samples	Plate Count at 95 F (35 C)		
		<200,000	210,000 to 1,000,000	>1,000,000
0.5	4	0%	0%	100%
1	15	0	0	100
2	47	0	6	94
3	51	6	22	73
4	56	25	38	38
5	47	47	43	11
6	48	73	25	2
7	33	85	12	3
8	25	84	12	4
9	12	83	8	8

When results obtained by the methylene blue test appear illogical on the basis of production methods used with the milk, microscopic or plate counts should be used as check tests. The methylene blue test does not give information about the probable cause of high bacterial counts. In instances where good sanitary control is maintained and bacterial counts are low, methylene blue tests are progressively less applicable.[31]

Resazurin Test

A search for other indicators that might be used in place of methylene blue in reduction tests led to the introduction of resazurin which imparts to fresh milk a characteristic blue color. Reduction of the dye is shown by a gradual change in color through various shades of purple and mauve to full pink, when apparently resazurin is completely converted to resorufin; in milk this stage of reduction is not reversible. A second stage of reduction consists of conversion of resorufin to dihydroresorufin, with a color shift from pink to white; this reduction is reversible.

By adding resazurin to milk, one type of bacterial action can be measured, just as when methylene blue is added. In general, the more rapid the changes in the dye, the higher the numbers of bacteria. Various factors influence the relationship, however.

Bases for Reading Test. With the resazurin test the results can be based either on (1) degree of color change after a stated incubation period, or (2) period required to reduce the dye to a given end point. A series of color standards is necessary for making readings on the former basis, and both Munsell color charts and comparator discs have been used. With the 1-hr test, the two systems compare as follows:

Class of Milk	Munsell Notation	Disc Reading
1 (Good)	PB 7/4 to PBP 7/5.5	4–6
2 (Fair)	PBP 7/5.5 to PRP 7/8	3
3 (Poor)	PRP 7/8 to colorless	1–2
4 (Very poor)	Colorless	0

Only one color standard is used for making readings on the latter basis, such as Munsell P 7/4 (midway between initial blue and pink) or disc reading 3. By taking readings at 3 successive hourly intervals, samples may be placed in any one of 4 classes. Because of the possibilities with the series of colors involved in the reduction of the dye, the test has been read on a number of different bases. Certain of them are considered to be more satisfactory than others, and methods for carrying them out have been standardized.[31]

Results Obtained with Test. Following work elsewhere, the resazurin test was introduced into the United States by Ramsdell *et al.*[59] They reported that, as used by them, the test could be completed in 1 hr and the milk classified into grades. Milk from diseased udders and physiologically abnormal milk had a significant effect on resazurin. Various additional investigations have shown (1) that results can be obtained more quickly with resazurin than with methylene blue, and (2) that certain samples of abnormal milk, due to physiological and pathological conditions (early lactation, advanced lactation, mastitis, etc.), are detectable with resazurin. The second point often is an advantage but is objected to by certain control officials who, when reduction tests are run, are interested primarily in the general bacteriological condition of the milk. For distinguishing changes in resazurin that are due to bacteria from those due to other causes, microscopic observations can be made.

Reading the resazurin test after 1 hr is most useful with relatively

poor milk supplies. However, some milk samples of this type contain many dormant bacteria and they produce little change in 1 hr. To detect such samples the incubation can be prolonged. Johns [60] noted that the 1-hr resazurin test does not indicate the bacterial content of milk as reliably as the methylene blue test, with inversion of the tubes, and places a larger proportion of high-count milk in the highest grade. He also reported [61] that with incubation until the pink color is reached resazurin tests compare very favorably with the methylene blue test, and the reduction period is shortened. Johns and Howson [62] found that resazurin reduces to the pink stage in approximately three-fourths the period required for methylene blue to reduce. The pink end point is of little value in detecting abnormal milk.

Bortree and Spencer [63] compared the results of the 30-min resazurin test and microscopic count on 916 samples of raw milk and of the 1-hr resazurin test and microscopic count on 1,020 samples. With the 30-min test, 93% of the samples placed in class 1 (Munsell PB 7/4) had microscopic counts of <200,000 per ml, and 3.5% were in the range of 200,000 to 400,000. With the 1-hr resazurin test, 94% of the samples in class 1 (Munsell PB 7/4 and PBP 7/5.5) had microscopic counts of <200,000 per ml. With both procedures, all samples placed in class 4 (decolorized) had high counts. Milk placed in classes 2 or 3 had both high and low counts.

Influence of Various Factors on Test. The resazurin test is influenced by many of the factors which influence the methylene blue test and some additional ones, such as leucocytes.

Variations in Reducing Ability of Bacteria. Pure cultures of bacteria vary markedly in their ability to reduce resazurin. Jones and Davis [64] noted that *Lactobacillus acidophilus* and *Bacillus subtilis* reduced resazurin more quickly than methylene blue, whereas *S. lactis, S. agalactiae, Streptococcus liquefaciens, Escherichia coli, Aerobacter aerogenes, Serratia marcescens,* and *Micrococcus roseus* reduced methylene blue more rapidly than resazurin. The difference was as much as 3 hr with *S. agalactiae. Micrococcus pyogenes* var. *aureus* reduced both dyes at about the same time. In general, bacteria which produce acid rapidly in milk also reduce resazurin rapidly.

Effect of Light. Frayer [54] found that, when exposed to bright sunlight, low-count milk to which resazurin was added behaved generally like a sample of poor milk. Thomas [65] noted that resazurin solutions prepared from either powder or tablets showed no change when held in the dark for 6 weeks at room temperature.

Effect of Low Temperature Storage of Samples. According to Revallier-Warffemius [66] storage of milk at 35.6 to 37.4 F for 24 hr caused more variation in the resazurin test, in comparison to tests on fresh milk, than in the methylene blue test. Such storage, however, improved the correlation between the two reduction tests because of inactivation of leucocytes at the lower temperature. Johns [67] conducted resazurin tests on samples of milk when fresh and after holding at 37.4 F for 2 hr and 23 hr. Resazurin reduction time was increased with low-temperature storage, especially by storage for 23 hr without dye added. Storage for 23 hr with dye added gave results which agreed quite well with 2 hr storage. Statistical analysis of the results showed no difference between the treatments, however. When it is difficult to conduct tests on the day of sampling, overnight storage in the presence of the dye was recommended. Other investigations [68, 69, 70, 71] indicate that resazurin reduction time is increased by storage of the samples at a low temperature.

Variations in Reading Tests. Philips and Soulides [72] found that, when 1 person tested 367 samples in quintuplicate, no differences were observed in 354 samples, and the maximum variation was half a disc number. When 2, 3, and 4 persons checked the samples, there was complete agreement in 85, 75, and 63% of the samples, respectively. In another experiment, 528 samples were tested in 4 separate laboratories and complete agreement was obtained with 78% of the samples; 3% of the samples gave variations of 4 disc numbers, or more. The greater variations in this experiment were attributed to the storage temperatures before testing.

Effect of Leucocytes. Thomas and Probert [73] found that increased leucocyte counts were associated with well-defined decreases in reduction time. Leucocyte counts of $<500{,}000$ per ml had little effect on reduction of resazurin or methylene blue, a count between 500,000 and 2,000,000 had an appreciable effect on resazurin but not much effect on methylene blue, and counts of $>2{,}000{,}000$ had a strong reducing effect on either indicator. Morgan and McKenzie [74] studied resazurin reduction using 211 samples of foremilk; 84 of the samples were mastitis positive on cultural examination. Of the samples, 78 reduced resazurin to below disc 4 in 1 hr when tested within 30 min after milking; 64 of the 78 samples, including 56 with leucocyte counts of $>1{,}000{,}000$ per ml, were mastitis positive. When the resazurin test was applied to the samples after holding for 24 hr at a low temperature, 45 reduced resazurin below disc 4 in 1 hr; 36 of these were mastitis positive and 35 contained $>1{,}000{,}000$ leucocytes per ml.

Value of Test. The resazurin test determines, in a general way, the bacteriological condition of raw milk. There is no close correlation between results obtained with it and with plate or microscopic counts, but it serves to detect milk supplies that need attention. Since results can be obtained more quickly with this test than with the methylene blue test, it often is preferred when the day's work is normally completed at a definite hour. Detection of abnormal milk also may be an advantage under certain conditions. The series of colors that should be distinguished in the resazurin test complicate it when the test is to be run by persons with relatively little training or with certain vision defects. Distinguishing between blue and white in the methylene blue test is very simple.

Golding and Jorgensen [75] noted that the resazurin test agrees reasonably well with the plate count and can be worked out so that a plant using the test can obtain about the quality of milk desired. Chilson and Collins [76] found that, as applied in their work, the resazurin test was a satisfactory and rapid criterion of both the initial quality and keeping quality of pasteurized 20 and 40% cream; it could not be depended on to detect high counts in cream that had been stored at 40 F, or lower, for 4 or more days.

The following data obtained by Black [58] on 339 samples of raw milk show the relation between resazurin reduction time and bacterial counts.

Percentage of Raw Milk Samples Falling into Various Plate Count Groups when Classified by Resazurin Reduction to the Munsell P 7/4 End Point

Reduction Time (hr)	Number of Samples	Plate Count at 95 F (35 C)		
		<200,000	210,000 to 1,000,000	>1,000,000
1	72	4%	4%	92%
2	104	15	33	51
3	80	55	33	13
4	83	84	13	2

Comparison of Methylene Blue and Resazurin Tests. Okulitch *et al.*[77] obtained about 80% correlation in comparisons of the methylene blue and resazurin tests. The plate and direct microscopic counts supported the resazurin test in more instances than the methylene blue test. Galesloot [78] determined the quality of 540 samples of winter milk and 184 samples of summer milk by the methylene blue and

resazurin tests. The agreement between the tests was very poor in about 66% of the winter samples, many of which had high catalase values, and the disagreement was considered due to leucocytes. Better agreement was obtained between the tests when samples were held cold for 16 hr, which apparently decreased the activity of leucocytes. Plate counts agreed better with the methylene blue than with the resazurin test. In the summer samples, leucocytes caused variations in about 25% of the samples. It was considered unlikely that all samples having a short reduction time were physiologically or pathologically abnormal since the catalase values were not abnormally high and occurrence of mastitis on farms was low.

Kastli and Binz [79] found that the 10- and 30-min resazurin tests failed to detect many milk samples which would have been rejected by the methylene blue test and microscopic colony count. The 2-hr resazurin test agreed well with these tests.

Influence of Penicillin in Milk on Dye Reduction. Johns and Katznelson [80] noted that dye reduction was retarded in all samples of milk containing 0.5 unit of penicillin per ml and in 1 sample containing 0.01 unit. It was estimated that mixed milk from a small herd in which 1 cow is treated with penicillin may contain as much as 0.8 unit per ml in the first and second milkings after treatment. If a resazurin triple-reading test is used, it is unlikely to be affected as the end point is reached before the retarding effect takes place. Low concentrations of penicillinase (0.63 unit per ml of dye-milk mixture) added to milk containing 0.1 or 0.2 unit of penicillin per ml completely counteracted the retarding effect of penicillin. Higher concentrations of penicillinase (2.1 units) increased the retarding action.

Hunter [81] found that with addition of 0.1 to 10 units of penicillin per ml of milk just prior to testing, 2 to 5 hr longer were required to decolorize methylene blue as compared to control samples. Penicillin concentrations of 0.1 to 0.5 unit per ml were as effective as higher concentrations. Lactic streptococci were more sensitive to penicillin than coliform types.

TESTS FOR CERTAIN TYPES OF ORGANISMS

The presence in milk or cream of relatively large numbers of organisms of a certain type sometimes permits more or less definite conclusions with reference to the products, and various tests involving this principle have been proposed. The organisms considered in such tests are objectionable because they may produce an undesirable

change, such as gas formation in cheese, or because they are associated with contamination from a specific source.

Wisconsin Curd Test

The Wisconsin curd test has been used primarily in the detection of milk that is unsatisfactory for cheesemaking; it shows especially the presence of gas-forming organisms but also may give information with reference to other undesirable types.

Method of Making the Test. Samples of milk to be examined are collected in sterile pint jars or wide-mouth bottles, the containers being almost filled. By means of a water bath, the samples are adjusted to about 90 F and 10 drops of rennet extract mixed with each. As soon as a firm curd is formed it is broken by shaking or cut into small pieces with a knife that has been sterilized or boiled. The container and curd are kept warm (90 F, or higher) to favor growth of the bacteria. As the whey collects it is poured off; the curd occasionally is broken by shaking or cutting to facilitate drainage of the whey. When a small pat of curd quite free of whey is obtained, the container is held at about 98.6 F. After 10 or 12 hr the curd is examined. If it is solid throughout, shows no sliminess at the surface, and has an agreeable odor, the milk probably was free of organisms which would be objectionable in cheesemaking, whereas gas holes in the curd, sliminess, objectionable odors, etc., show the presence of organisms which probably would cause undesirable conditions in cheese; in some instances the curd is so full of gas holes that it is spongy. Milk with a high bacterial count may give a desirable curd and another lot with a low count may give an undesirable curd so that the test is of no value in comparing samples of milk from the standpoint of their keeping qualities.

Value of Test. Use of the test on milk from individual farms makes it possible to detect lots that are objectionable for cheese manufacture. Although results of the test are not available before the cheesemaking is begun, there ordinarily is a general similarity in the milk from a farm so that the farms needing improvements in methods of milk production can be detected. The test is of less importance than formerly because extensive use of pasteurized milk has aided greatly in the control of objectionable fermentations in various cheeses.

Anaerobic Spore Test

Some of the spore-forming, anaerobic bacteria produce a "stormy" or gassy fermentation in milk that is more or less characteristic, and

certain investigators have attempted to establish the significance of this change in milk held under conditions favorable for it. Weinzirl and Veldee [82] called attention to the possibility of testing milk for contamination with manure in this way. The procedure involved heating milk in definite amounts, such as 5, 10, and 15 ml, to 176 F for 10 min to kill the nonspore-forming bacteria that might interfere with the fermentation, covering with sterile paraffin to a depth of about ⅛ in., and then cooling and incubating at 98.6 F. If certain anaerobes are present, gas will be formed and will force up the paraffin plug. The investigators considered that determination of the amount of milk required to give a gassy fermentation, under the conditions used, made it possible to obtain information on the extent of contamination with manure. Later, Weinzirl [83] used the following procedure: 0.5 to 1 ml of melted paraffin is placed in 1.5-cm test tubes which are plugged with cotton and sterilized, with either dry or moist heat. By means of a sterile pipette, 5 ml of the milk to be tested is placed in each of 5 tubes, and the tubes then are heated in an Arnold sterilizer to 176 F for 10 or 15 min. The melted paraffin rises to the surface where it hardens when the temperature is lowered. The tubes are incubated for 3 days at 98.6 F. If 2 tubes out of the 5 form gas, the milk is considered to show excessive contamination.

In the studies of Ayers and Clemmer,[84] 10 tubes of milk, each containing 20 ml, were used per sample and there was a fairly definite relationship between the results and the conditions of production; but it was noted that no reliance could be placed on 1 test and that tests on a number of samples were necessary for the results to have significance.

Hudson and Tanner [85] compared the anaerobic spore test with the microscopic colony count and the sediment test on >100 samples of milk. They concluded that the procedure cannot be accepted as a method of accurately determining manurial pollution of milk; no correlation was found between results of the test and numbers of bacteria or amounts of dirt.

The anaerobe of most importance in causing gas in heated milk held in deep layers appears to be *Clostridium sporogenes* but, undoubtedly, other species also are involved. Results [84] indicate that the majority of the spores of *Cl. sporogenes* gains entrance to milk from cow manure. However, there are various other sources from which spores of anaerobes can enter milk.

Tests for *Escherichia-Aerobacter* Organisms

The *Escherichia-Aerobacter*, or coliform, group of organisms includes the aerobic and facultative anaerobic, gram-negative, nonspore-forming bacteria which ferment lactose with formation of gas. The importance of certain of these organisms as an indication of the bacteriological condition of water supplies has led to attempts to use them in determining the quality of milk. Because of the various opportunities for the contamination of milk with the organisms and also for their growth in milk, conclusions with reference to them can be drawn only under specific conditions. Milk produced with excellent procedures ordinarily contains some of the organisms although it may be necessary to examine relatively large volumes to detect them.

Coliform organisms gain entrance to milk from a variety of sources, including manure, dirt, utensils in poor bacteriological condition, the milker, flies, etc., and, occasionally, from infected udders. Griffin and Stuart [86] noted that the distribution of 6,577 strains of coliform organisms from milk, water, soil, grains, and feces indicated that *Aerobacter* and intermediate organisms constitute the normal coliform flora of nonfecal materials, while *Escherichia* organisms are normal to feces. They suggested that occurrence of these groups outside the usual habitats is adventitious.

When once in milk, coliform organisms may multiply actively if the conditions permit. *Aerobacter* types multiply at lower temperatures than the *Escherichia* types as would be expected from the sources of the two groups. Robinton and Genung [87] reported that coliform organisms develop much more rapidly in cream than in milk, and the former apparently contains either some accelerating growth factor or a greater surface area of available food materials.

Significance. Sherman and Wing [88] studied the application of a coliform standard of 1,000 per ml to 220 samples of milk before and after holding and found that about ⅔ of the initially satisfactory milk that was held at either 50 F for 48 hr, or 59 F for 24 hr, would have been condemned on the basis of containing >1,000 coliform organisms per ml. Of those held at 50 F for 24 hr, 13% would have been condemned. The investigators concluded that the coliform test is of no special value as an index to the sanitary conditions surrounding the production of raw milk of the usual grade; in the case of high-grade milk containing <10,000 total bacteria per ml the test may have a place as a supplementary index of quality. They noted that if all

milk was cooled promptly to 45 F and maintained at that temperature, the test might prove of more general value.

Yale and Eglinton [89] concluded that the coliform test has only slight value as a routine test for ordinary fresh milk because of the time required to make the observations necessary to determine which of the various causes of high coliform counts is at fault; high counts usually are not due to manurial contamination but to growth on utensils and in milk. They found that growth in milk during the 2-hr interval between production on the farm and delivery to the plant increased the count from 4 to 52 per ml with 1 can of milk and from 1,300 to 6,200 with a second can.

Relatively large numbers of coliform organisms in a lot of milk definitely suggest an undesirable condition, just as do relatively large numbers of other groups of organisms or of total bacteria. An undesirable condition may involve methods of production and/or methods of holding. Commonly, the objectionable condition also is evident from total bacterial counts; in some instances an irregularity in the methods is shown by total counts when it is not shown by coliform estimations.

In dealing with a milk supply normally having low bacterial counts, contamination from certain sources may be more evident from coliform counts than from total bacterial counts, and with raw milk supplies intended to be of excellent bacteriological quality, coliform counts may be an additional protection beyond that given by total counts. When they are used, it is advisable to determine whether representative cultures are *Escherichia, Aerobacter,* or intermediate types. Stuart *et al.*[90] found that the coliform organisms from certified milk showed considerable variation in the proportion of the three types, with a marked predominance of *Aerobacter* and intermediate forms; these would be less objectionable than *Escherichia* types, although still undesirable.

Tests for Specific Pathogens

The most important information with reference to milk, cream, ice cream, butter, cheese, or any other food product is whether it contains pathogenic organisms. The difficulties in detecting such organisms in dairy products are so great that tests for them rarely are used in a routine way. Methods of examination for certain pathogens, notably *Mycobacterium tuberculosis* and *Brucella* species, yield accurate results, but the methods for others are so unsatisfactory that negative findings do not admit of conclusions. The methods that are satisfac-

tory are limited in their usefulness by the long periods required before the results are available or by the small volumes of milk that can be examined. Moreover, the absence of a particular pathogen in milk from a certain source on one day is no assurance that it will continue to be absent. Because of the number of pathogenic species that are spread through milk, an examination for each involves an excessive amount of work that is not justified in view of the inadequacy of many of the methods and the limited conclusions that can be drawn.

TESTS FOR UDDER INFECTIONS

Udder infections are widespread among dairy cows. The organism most commonly responsible is *S. agalactiae*, and it is nonpathogenic for man but, occasionally, the causative organism is a species that can produce disease in man. From the aesthetic standpoint, use of milk from infected udders is objectionable. Control of udder infections in herds also is advisable because of the economic losses involved in decreased milk production, rejected milk, etc. Maintenance of herds free from udder infections is receiving much attention.

The detection of udder infections is complicated by two factors: (1) The infection may be very slight so that the secreting tissue is only slightly abnormal, and (2) there may be only a small portion of a quarter involved and milk from this is mixed with a much larger volume of milk from normal tissue. If milk from an infected quarter is mixed with milk from normal quarters, the problem is still further complicated, and it is advisable to examine milk from individual quarters; there also is an advantage in using milk soon after it is drawn so that changes due to bacterial action are excluded.

Various tests are applied to milk in an attempt to detect irregularities which indicate that it came from an abnormal udder. In some cases results of a test admit of definite conclusions but in others they do not, and results of a combination of tests are advisable. Moreover, udders which are normal at one time may be infected later so that tests at intervals are desirable in any attempt to control udder infections.

Physical Examination of the Udder. Many cases of udder infection can be detected by physical examinations of udders by a person familiar with udder anatomy. Such an examination is made most advantageously immediately after milking when the udder is not distended with milk. By careful examination of each quarter of the udder by manual palpation, induration or fibrosis may be detected.

Infected udders generally show one or more hardened areas, and the quarters may lack uniformity in size and consistency. Udall and Johnson [91] outlined a procedure for physical examinations of udders based on symmetry and consistency. At times, it is difficult to interpret the cause of scar tissue or indurations, and tests which can be applied to milk may be useful in the interpretation.

Appearance of Milk. Milk from an infected udder sometimes is abnormal in appearance. It may be watery, flaky, etc., and the color may be abnormal. Drawing the first few streams of milk from a quarter into a cup lined with fine wire screen or black cloth, or onto a dark-surfaced metal or plastic, aids in detecting certain abnormal conditions. Use of a strip cup frequently is a part of each milking operation, especially in the production of milk of fine quality.

Acidity. In certain cases of udder infection, the acidity of the milk is decreased, presumably due to infiltration of blood serum. Rosell [92] considers that if the acidity of milk from 1 quarter is less than 0.10% and that from other quarters is higher, mastitis probably is indicated.

A colorimetric *p*H test involving use of bromthymol blue is extensively employed. It consists of adding the indicator to a small amount of milk and observing the color. Hucker *et al.*[93] suggested 2 drops of a concentrated alcohol solution to 3 ml of milk, and other investigators employ various concentrations. Within reasonable limits, concentration apparently makes little difference, but the indicator solution should be adjusted to approximately the neutral point by addition of sodium hydroxide. A yellowish-green to greenish-yellow denotes a normal reaction, a bluish-green a suspicious reaction, and a blue color a decidedly positive reaction. In some cases paper impregnated with bromthymol blue has been used for the test; milk is dropped on the paper or the paper dipped into the milk. Bromcresol purple also has been used as an indicator for determining the *p*H of milk.

A reaction distinctly more alkaline than normal milk indicates an udder infection, but a negative test is no assurance that the udder is normal. Shaw *et al.*[94] considered the *p*H test extremely unreliable in detecting cows with chronic mastitis. Fay *et al.*[95] reported the bromthymol blue test detected only 21.1% of samples from quarters known to be infected. In certain cases milk from an infected udder is more acid than normal milk.

Catalase. The catalase content of milk from infected udders often is higher than normal, the high value evidently being associated with a high content of leucocytes. Different methods of determining the catalase content of milk are used; in general, they consist of adding

hydrogen peroxide to milk, collecting the liberated oxygen in a tube, such as a fermentation tube, and measuring it. Various standards have been employed in interpreting results of catalase tests.[96] In general, a catalase content distinctly higher than normal indicates an udder infection, but milk from certain abnormal udders does not contain an excessive amount of the enzyme. Although some bacteria produce catalase, they are of no importance when fresh milk is studied.

Chlorides. In cases of udder infections the chloride content of milk often is high. Various procedures have been used to determine the chlorides in milk, and various standards have been proposed; some of the titration methods give values that are higher than the actual chloride content, as determined on an absolute basis.

Sharp and Struble [97] noted that the chloride content of the milk of healthy cows fell very rapidly during the first few days of lactation, reached a minimum, increased slightly during the first 60% of the lactation period, and then increased more rapidly, particularly during the last 10%. A definite relation was found between the chloride value and the production of milk by healthy cows; an increase in the chloride content began when the production of an animal fell below certain limits. In the case of milk from infected quarters, the first and last milk was high in chlorides, whereas the middle portion often approached or fell within the range of milk from normal udders.

The value of chloride determinations in detecting milk from infected udders is limited because of the other influencing factors, and some investigators consider it very unreliable.

Lactose. In milk from infected udders the lactose content, as determined with the usual chemical methods, often is low. A lactose content under 4% sometimes is considered to indicate an abnormal udder. Rosell [92] reported milk which contained no lactose.

Body Cells. Milk normally contains cells that come from the body of the producing animal. Some are contributed by the secreting tissue, some by the linings of the milk ducts, milk cistern, and teat canal, and some by the surface of the teat and udder, but the majority are cells that, because of their general appearance, commonly are considered to be leucocytes. The leucocytes are largely of the polymorphonuclear type, but other types also occur; occasionally, cells containing bacteria are observed.

Excessive numbers of body cells often are associated with udder infections. However, the relationship between abnormal udder conditions and numbers of body cells in the milk is not a definite one, and cell determinations alone may permit only limited conclusions

although they are valuable when used to supplement other evidence. Before the variations that occur in the numbers of cells in normal milk were recognized, milk from excellent dairies occasionally was excluded from certain cities because the cell counts were believed to indicate abnormal udders.

Methods for Body Cell Counts. Various methods have been used to determine the numbers of body cells in milk. Some of the earlier methods consisted of concentrating the cells by centrifuging and then counting them in a definite volume of the concentrate [98,99] or reading the percentage of sediment directly by use of a special centrifuge tube which is drawn out at the bottom to a fine bore and calibrated.[100] The procedure for the microscopic count of bacteria in milk is applicable to counting body cells and largely has superseded the other procedures. The general method was first employed for this purpose by Prescott and Breed [101] who pointed out that when milk is centrifuged many of the cells are carried into the cream, and that the percentage of cells thrown into the sediment is extremely variable.

Number of Cells in Normal Milk. Milk from different quarters of a normal udder shows considerable variation in body cell content, and there also is a variation from day to day in the cell content of milk from a given quarter. The numbers of cells which various investigators have considered the maximum for normal milk differ greatly, and over the years there has been a lowering of the standard. At one time counts of 1, or more, million per ml were regarded as normal by certain investigators. A standard of 500,000 per ml now is used in many cases when applied to milk from individual quarters. Other standards also have been suggested.[92,102]

MacLeod *et al.*[103] determined the relationship between the cell count of herd milk, as received for processing, and the incidence of mastitis in the herd. The results suggested that, if a herd had $<15\%$ infection, the leucocyte count of the herd milk may be affected by factors other than the number of animals with mastitis (e.g., the proportion of cows in early or late lactation), whereas above the 15% level the percentage of herd infection may be the important factor contributing to the cell count. The average leucocyte count of milk from infection-free herds appeared to be approximately 750,000 cells per ml. Where the level of infection within a herd was $>40\%$, the mean cell count of the herd milk could be expected to be $>1{,}000{,}000$ per ml. In general, infection due to *S. agalactiae* gave higher cell counts than infections due to other organisms.

Variations in Different Portions of a Milking. The different portions of a milking show variations in the cell counts that often are pronounced. In general, strippings run considerably higher than other portions of the milking, and foremilk may be either higher or lower than middle milk. In 15 comparisons Ross [104] found that, on the average, strippings had a cell content 53.85% greater than foremilk and 52.14% greater than middle milk. Russell and Hoffmann [105] studied 18 animals and noted that, on the average, strippings contained three times as many cells as foremilk or middle milk. In the results reported by Campbell,[106] strippings usually showed the highest cell count, although occasionally foremilk was high.

Kesler *et al.*[107] conducted analyses on milk drawn from the udder at different periods during milking to determine the most satisfactory method of sample selection. The first, second, and third 15-ml portions were quite uniform in leucocytes, the midmilk was somewhat higher, and the strippings highest. The authors suggested that the first or second 15-ml portion would be a satisfactory sample. Long-chained streptococci were more numerous in foremilk than in midmilk or strippings.

The higher count obtained with strippings than with other portions of a milking may be due to a tendency for the manipulation of the udder during the stripping process to dislodge cells so that they are more easily carried out with the milk.

Influence of Stage of Lactation. Colostrum commonly has a higher cell content than the normal milk secreted later. The following cell counts on colostrum and normal milk, respectively, taken from the studies of Ross,[104] illustrate this relationship: 713,500 and 18,000 per ml; 3,935,000 and 13,000; 500,000 and 58,000. Ross stated that, "Apparently most of the cells present in colostrum are broken-down epithelial cells," whereas Breed [108] reported that, "No cells of a different type than those formed in later stages of lactation were found in the colostral milk."

MacLeod and Anderson [109] found that the geometric average cell count of milk from healthy cows, beginning with the second week through the forty-first week after calving, was approximately 70,000 per ml. There was no trend in the counts on the milk of 10 cows studied as a group, but the mean counts on the milk of individual animals showed significant trends when compared with each other. A downward trend was apparent in the weekly mean counts through the second to the fourth week after parturition considering the group as a whole, but after the fourth week there was no noticeable or con-

sistent change evident through the forty-first week. The cell counts of milk from 20 healthy cows from the first to the twenty-first day after calving were determined to establish the time after calving at which the cell counts might be expected to approximate the mean cell count of normal milk. The overall average cell count was 150,000 per ml, that for the first week after parturition was 430,000, that for the second week was 100,000, and that for the third week was 70,000. It was concluded that the cell count of milk may be expected to return to a normal level between the second and fourth week after calving. On the first day after calving, the mean cell count of milk may be expected to be well over 1,000,000 per ml. A small, but significant, difference was noted in cell counts of morning and evening milk, the evening count being slightly higher.

Influence of Various Factors. Results obtained by Russell and Hoffmann [105] showed a marked tendency for the cell content of milk to increase with an increase in the age of the cow. This may have been due to a greater opportunity for development of abnormal udder conditions with the animals that had been through a number of lactation periods.

In studies on the effect of various methods of drying up cows, Wayne and Macy [110] found that incomplete, intermittent, and incomplete intermittent milking, as well as complete cessation of milking, did not appear to have any marked effect on the cell content of the milk in subsequent lactation periods. Incomplete and intermittent milking usually resulted in somewhat higher cell counts during the periods of use. The cells observed in the milk during the drying up process were large, mononuclear cells. Anderson and MacLeod [111] noted that when the milking of mastitis-free cows in midlactation was interrupted for 10 days, the average cell count on resumption of milking was approximately 27 times higher than before the interruption.

It appears that machine-drawn milk sometimes has higher cell counts than comparable hand-drawn milk; this may be related to leaving the machines on the cows for excessive periods. Hucker [112] reported that milk from cows milked by machine gave an increased number of cells over comparable milk from cows milked by hand. Cone [113] found that, under the conditions used, milk from most of 31 cows had higher cell counts and also chloride values when the animals were machine milked than when they were hand milked; this was true with both infected and noninfected quarters. There was a marked difference between cows in their response to machine milking. During the investigation, little attention was given to the periods the

machines were left operating, although the general tendency was to remove them as soon as the milk flow ceased.

Watts [114] noted that a number of herds milked with one particular type of milking machine had an average mastitis incidence of 45%. When another machine was used, the infection rate dropped to the average (about 20%) for herds using this machine. A herd which continued to use the former machine had a mastitis incidence of about 64%. Meigs *et al.*[115] found that the incidence and severity of mastitis was related closely to the amount of vacuum and length of time the teat cups were left on the udder. Considerable improvement resulted when cows with mastitis were changed from routine, or severe, to mild machine milking and there was particularly marked improvement, or almost complete recovery, when they were changed to hand milking. When previously mastitic cows milked by the routine method were changed to mild machine or hand milking, the milk yield tended to return to the normal level and the number of leucocytes, number of streptococci, and per cent chlorides in the milk decreased markedly. An abnormally high percentage of chlorides usually was present in the milk from normal cows on routine milking even though the animals never became mastitic.

Influence of Condition of Udder. An infection of the udder is the important factor causing a high cell content in milk. Ross [104] noted an instance in which a cow developed an abnormal quarter and milk from this had a cell count of 62,400,000 per ml, while that from the other 3 quarters contained 407,000. Russell and Hoffmann [105] reported results of cell counts on milk from 3 cows, each of which had an abnormal udder during part of the examination period. The highest cell count obtained with milk that showed evidence of mastitis was 3,625,000 per ml and the lowest was 489,000. Cherrington *et al.*[102] noted that the average cell count on milk from 6 normal cows was 43,000 per ml, whereas that on milk from 7 diseased cows was 3,000,000; the maximum count on milk from individual quarters was 37,400,000 per ml. Many other examples of high cell counts on milk from diseased udders have been reported.

When milk has an excessive cell count it ordinarily has come from an infected udder. However, in some instances milk from an abnormal udder does not have a high cell count and the numbers may even be relatively low. Interpretation of intermediate cell counts presents difficulties, and various other tests often are used to supplement the cell counts in an attempt to establish the condition of the udder.

Examinations for streptococci are of special importance in this connection.

Hoffmann [116] found that the injection of such inert materials as physiological salt solution, boric acid solution, and distilled water produced a rapid and marked leucocytosis, as shown by swelling and fever of the quarters injected and by increased cell content of the milk. A considerable number of the samples of milk showing high cell counts were reported as abnormal, but abnormal milk also gave counts that could not be considered excessive and it appeared that there is no close relationship between the cell content and an abnormal condition in the milk. The highest count reported by Hoffmann in the work on experimental leucocytosis was 25,400,000 per ml; this was obtained on abnormal milk coming from a quarter that had been injected the previous day with 300 ml of sterile distilled water.

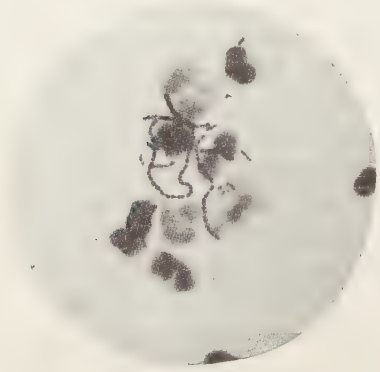


Figure 3. Streptococci and leucocytes in milk from an abnormal udder.

Influence of Clarification and Separation on Cells. Clarification or separation removes a portion of the cells in milk (Chapter 7). This effect must be recognized when cell counts are made on milk that has been handled in a plant, since the number of cells originally present may have been reduced materially. Occasionally, a farmer reports that the amount of slime collecting in the separator bowl suddenly is increased; most often this is due to an excessive number of cells in the milk as a result of one or more udder infections.

Streptococci. Because of the frequency with which udder infections are due to streptococci, milk sometimes is examined microscopically for this morphologic type. Large numbers in freshly drawn milk, or in milk held so that there has been no bacterial growth in it, are indicative of udder infections, especially when accompanied by high cell counts; under these conditions the streptococci commonly are in long chains. Samples of milk from infected udders sometimes can be recognized immediately by the combination of long-chain streptococci and many cells in microscopic preparations.

Because streptococci causing mastitis may be present in milk in

small numbers, milk sometimes is incubated 12 to 24 hr at 98.6 F (37 C) and examined microscopically. With this procedure streptococci may multiply and be detected easily. If the milk is collected aseptically there is little danger of contaminants leading to erroneous conclusions, but if the results are in question it is advisable to isolate and identify the streptococci which have multiplied. In some cases streptococci are present in normal udders; since these may include S. *agalactiae*, results must be interpreted with care. Some investigators have added certain chemicals to milk samples to be incubated with the idea of controlling growth of contaminants.

Plating of samples of milk, usually on blood agar, is another method of examining milk for streptococci. The procedure often is followed when a milk supply appears to be involved in the spread of septic sore throat, scarlet fever, or some other disease associated with streptococci. It also can be used in examining milk for S. *agalactiae*.

It should be recognized that mastitis may be due to organisms other than streptococci; these include staphylococci as well as organisms of types much less commonly related to infections so that failure to find streptococci is no assurance that the udder is normal.

Hotis Test. The Hotis test was described by Hotis and Miller [117] as a rapid means of detecting S. *agalactiae*. More recently the importance of other organisms as a cause of mastitis has been recognized and some of them also can be shown by the test.[118]

In the test, 9.5 ml of milk, which should be collected with all precautions against contamination, is mixed with 0.5 ml of sterile 0.5% bromcresol purple in a sterile test tube having an outside diameter no greater than 15 mm. The sample is incubated 20 to 24 hr at 98.6 F (37 C); longer incubation does not improve the results materially and may allow small numbers of contaminating bacteria to increase and interfere with the test. The fresh mixture of dye and milk normally has a purple color. If, due to mastitis, the milk is more alkaline, the original color of the mixture shows this. Cone and Grant [119] noted the importance of the original color of the mixture in showing irregularities in milk.

After incubation, the color of the mixture may be unchanged or it may show various conditions as a result of the growth of organisms causing mastitis. With S. *agalactiae* there commonly is the formation of yellow flakes or clumps along the sides of the tubes and often a coarse flocculent sediment; the color of the milk also is changed as a result of acid formation. Other organisms produce changes of various

types that may be rather characteristic. Rust-colored colonies are indicative of *M. pyogenes* var. *aureus*.

To avoid action of contaminating organisms in obscuring positive results with the test, certain chemicals have been added to the milk or the dye solution. With sodium azide, equal volumes of 0.2% aqueous solution and 0.5% solution of bromcresol purple are mixed and 0.5 ml of the mixture added to 9.5 ml milk. Miller [118] noted that, with azide, growth of contaminants in Hotis tests was greatly repressed for at least 48 hr, and streptococci continued to grow. However, the changes were not always typical and 48-hr incubation may be required for positive results. Also, staphylococci do not grow well in the presence of the azide, so that some of the information that might be obtained with the test is lost when the azide is used.

Miller [118] reported that the test was 85 to 90% as effective as cultures on blood agar plates in detecting *S. agalactiae* in milk, but only a small percentage of milk samples containing *Streptococcus equisimilis* and *Streptococcus uberis* were positive. *M. pyogenes* var. *aureus* was identified readily.

Frayer [120] conducted Hotis tests on 796 samples of milk in which streptococci, with or without excessive leucocytes, were found. The tests were carried out with only bromcresol purple added and also with the indicator plus preservative (sodium azide, etc.). Yellow flakes were present in 700 samples by the former method and in 200 by the latter method. A heavy yellow sediment plus a yellow cream line, or a yellow cream line alone, was noted in 75 samples tested by the former method and in 585 tested by the latter method. No reaction occurred in 21 samples tested by the former method and 11 tested by the latter method.

Whiteside Test. Whiteside [121] found that on addition of 2 ml of *N*/1 sodium hydroxide to 10 ml of milk from a cow having mastitis, with subsequent beating of the mixture with a glass rod, a viscid mass was formed. The reaction took place immediately at room temperature but failed if the mixture was brought to the boiling point. The test was positive before the bromthymol blue and strip cup tests and was most marked during the acute stage of the infection.

Murphy and Hanson [122] modified the test. They used 5 drops of foremilk and 1 drop of *N*/1 sodium hydroxide on a flat glass plate with a dull green blotter as a background. After mixing for 20 sec with a glass rod, the material is examined by reflected light for the presence of a precipitate. Amount and character of the precipitate can be graded. The test paralleled closely the leucocyte count in ability to

detect the presence of udder infections. The authors consider it to be a simple, accurate, presumptive test for the presence of chronic mastitis.

Dunn *et al.*[123] concluded that leucocytes are directly or indirectly responsible for the Whiteside reaction in mastitic milk. They postulated that the protein material of the leucocytes reacts with sodium hydroxide to form a gelatinous mass.

Curd Tension. Although curd tension determinations ordinarily are not used in attempts to detect mastitis, the effect of mastitis on the curd tension of milk is of interest because soft curd milk may be desired for infants and invalids.[124]

Mastitis, including subclinical mastitis, may cause a decrease in curd tension of milk from the infected quarter or quarters. Extent of the drop may vary with the degree of the infection. Effect of mastitis on curd tension of milk is shown by comparisons of milk from normal and infected quarters of the same cow. Hansen *et al.*[125] noted that mastitis caused by streptococci invariably lowered the curd tension of milk but that mastitis due to micrococci apparently had no appreciable influence on it. Anderson *et al.*[126] reported that the three principal groups of organisms associated with chronic mastitis (two types of streptococci and micrococci) were about equally effective in reducing curd tension, but that coliform infections decreased the curd tension more than any other type. According to Sanders *et al.*,[127] mastitis caused a decrease in curd tension. There was a tendency for the properties of mastitis milk to return to normal when the bacteriological and pathological evidence of the infection subsided.

The general results indicate that in the selection of soft curd milk care should be taken to avoid cows with mastitis.

Comparison of Diagnostic Methods. Milk samples and udder tissue were studied by Chu [128] to compare indirect diagnostic tests for mastitis with culture tests (morphological and biochemical) and histopathological examinations. When compared with the culture tests, the chloride, catalase, Whiteside, and cell count tests agreed in only 50.6, 46.0, 49.4, and 45.0% of the cases, respectively. When compared with histopathological examinations, the corresponding percentages were 87, 92, 82, and 100. The culture tests failed to identify >45% of the mastitis cases detected by histopathological examination of udder tissue.

Malcolm and Campbell [129] found that in routine or research work the cell content and electrical conductivity tests are of great diagnostic value, provided colostrum and late lactation milk are excluded.

Various tests for the detection of mastitis were compared by Merilan *et al.*[130] using a microscopic examination and the Hotis test as standards. A leucocyte count of >500,000 per ml was in agreement with the standards in 71.9% of 203 infected quarters and gave 16% false positives. A chloride content of 0.14% detected 53.5% of 1,299 positive quarters and gave 21.6% false positives. The production of 1.5 ml of gas in the catalase test detected 45.47% of 552 positive quarters and gave 11.3% false positives. The bromthymol blue test showed agreement in 33.1% of 535 positive quarters and gave 8.9% false positives. The *p*H of milk, determined electrometrically, detected only 18.3% of 274 positive quarters and gave 8.1% false positives.

Frayer [120] found that composite herd samples failed to detect mastitis in 12 of 80 herds examined; however, 11 of the 12 herds had leucocyte counts of >700,000 per ml and were called doubtful. With 1,054 composite udder samples, 48 infected cows were not detected but 40 of the 48 were classed as doubtful. In a study of 100 individual quarter samples, it was noted that the first 2-ml portion of milk disclosed only 1 case of infection that was not found also in the second and third 10-ml portions. Only 4 of 100 positive samples showed microorganisms without incubation of the samples.

REFERENCES

1. *Wis. Agr. Expt. Sta. 15th Ann. Rept.*, 104 (1898).
2. *Mich. Agr. Expt. Sta. Tech. Bull.* 181 (1943).
3. *J. Dairy Sci.*, **21**, 661 (1938).
4. *Calif. Agr. Expt. Sta. Bull.* 595 (1935).
5. *U. S. Dept. Agr. Bull.* 1326 (1925).
6. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 155 (1913).
7. *J. Dairy Sci.*, **5**, 201 (1922).
8. *Ia. Agr. Expt. Sta. Research Bull.* 64 (1920).
9. *J. Dairy Sci.*, **11**, 375 (1928).
10. *J. Biol. Chem.*, **40**, 137 (1919).
11. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 70 (1919).
12. *J. Dairy Sci.*, **3**, 227 (1920).
13. *J. Dairy Sci.*, **7**, 468 (1924).
14. *Can. J. Research*, **24**, 5 (1946).
15. *J. Dairy Research*, **2**, 179 (1931).
16. *Wis. Agr. Expt. Sta. Research Bull.* 127 (1935).
17. *J. Dairy Sci.*, **19**, 235 (1936).
18. *Conn. (Storrs) Agr. Expt. Sta. 16th Ann. Rept.*, 150 (1904).
19. *J. Dairy Sci.*, **4**, 7 (1921).
20. *J. Dairy Sci.*, **7**, 86 (1924).
21. *U. S. Dept. Agr. Bull.* 944 (1921).
22. *J. Dairy Sci.*, **6**, 176 (1923).
23. *U. S. Dept. Agr. Bull.* 202 (1915).
24. *J. Dairy Sci.*, **6**, 95 (1923).
25. *J. Bacteriol.*, **15**, 93 (1928).
26. *Australian Milk Dairy Products J.*, **16** (11), 7 (1949).
27. *Milchwissenschaft*, **4**, 245 (1949).
28. *J. Dairy Research*, **14**, 21 (1945).
29. *Australian J. Dairy Technol.*, **7**, 10 (1952).
30. *J. Soc. Dairy Technol.*, **2**, 149 (1949).
31. *Standard Methods for the Examination of Dairy Products* (last

ed.). American Public Health Association, Inc., New York, N. Y.
32. *Am. J. Public Health,* **25,** 1114 (1935).
33. *Dairy Inds.,* **16,** 143 (1951).
34. *J. Dairy Research,* **10,** 35 (1939).
35. *Indian J. Dairy Sci.,* **4,** 103 (1951).
36. *J. Dairy Sci.,* **22,** 209 (1939).
37. *Vt. Agr. Expt. Sta. Bull.* 312 (1930).
38. *Vt. Agr. Expt. Sta. Bull.* 374 (1934).
39. *Med. Research Council Special Rept.* 206 (1935).
40. *J. Milk and Food Technol.,* **15,** 8 (1952).
41. *J. Dairy Research,* **16,** 310 (1949).
42. *J. Dairy Sci.,* **20,** 693 (1937).
43. *Vt. Agr. Expt. Sta. Bull.* 424 (1937).
44. *J. Milk Technol.,* **8,** 67 (1945).
45. *J. Dairy Sci.,* **5,** 438 (1922).
46. *J. Agr. Research,* **40,** 855 (1930).
47. *J. Agr. Research,* **44,** 85 (1932).
48. *J. Dairy Research,* **7,** 31 (1936).
49. *Kgl. Lantbrucks-Högskol. Ann.,* **17,** 361 (1951).
50. *Welsh J. Agr.,* **16,** 231 (1940).
51. *J. Dairy Research,* **10,** 88 (1939).
52. *J. Bacteriol.,* **18,** 293 (1929).
53. *Food Manuf.,* **6,** 196 (1939).
54. *Vt. Agr. Expt. Sta. Bull.* 498 (1942).
55. *Intern. Dairy Congr., Proc. 12th Congr.,* **2,** 484 (1949).
56. *J. Dairy Sci.,* **13,** 221 (1930).
57. *Quart. J. Agr. Sci.,* **2,** 128 (1945).
58. *J. Milk and Food Technol.,* **11,** 5 (1948).
59. *J. Dairy Sci.,* **18,** 705 (1935).
60. *Am. J. Public Health,* **29,** 239 (1939).
61. *Am. J. Public Health,* **33,** 393 (1943).
62. *J. Dairy Sci.,* **23,** 295 (1940).
63. *J. Milk and Food Technol.,* **11,** 255 (1948).
64. *Proc. Soc. Agr. Bacteriologists,* 38 (1944).
65. *Proc. Soc. Agr. Bacteriologists,* 46 (1943).
66. *Neth. Milk Dairy J.,* **1,** 152 (1947).
67. *J. Milk and Food Technol.,* **15,** 8 (1952).
68. *J. Dairy Research,* **16,** 310 (1949).
69. *Verslag. Landbouwk. Onderzoek.* 52 (1946).
70. *Proc. Soc. Agr. Bacteriologists,* 1 (1941).
71. *Vt. Agr. Expt. Sta. Bull.* 435 (1938).
72. *Proc. Soc. Agr. Bacteriologists,* 50 (1943).
73. *Dairy Inds.,* **6,** 295 (1941).
74. *Proc. Soc. Agr. Bacteriologists,* 64 (1943).
75. *J. Milk Technol.,* **8,** 189 (1945).
76. *J. Milk Technol.,* **3,** 334 (1940).
77. *Can. Dairy Ice Cream J.,* **25** (11), 3 (1946).
78. *Neth. Milk Dairy J.,* **2,** 70 (1948).
79. *Schweiz. Milchztg.,* **76,** 437 (1950).
80. *J. Milk and Food Technol.,* **12,** 133 (1949).
81. *J. Dairy Research,* **16,** 149 (1949).
82. *Am. J. Public Health,* **5,** 862 (1915).
83. *Am. J. Public Health,* **11,** 149 (1921).
84. *U. S. Dept. Agr. Bull.* 940 (1921).
85. *J. Dairy Sci.,* **5,** 377 (1922).
86. *J. Bacteriol.,* **40,** 83 (1940).
87. *J. Milk Technol.,* **8,** 97 (1945).
88. *J. Dairy Sci.,* **16,** 165 (1933).
89. *Intern. Assoc. Dairy and Milk Inspectors, 24th Ann. Rept.,* 116 (1935).
90. *J. Bacteriol.,* **36,** 411 (1938).
91. *Cornell Vet.,* **21,** 190 (1931).
92. *Sci. Agr.,* **15,** 169 (1934).
93. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 199 (1932).

94. *J. Dairy Sci.*, **20**, 199 (1937).
95. *J. Dairy Sci.*, **20**, 442 (1937).
96. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 626 (1933)
97. *J. Dairy Sci.*, **18**, 527 (1935).
98. *Md. Agr. Expt. Sta. Bull.* 102 (1905).
99. *Standard Methods of Milk Analysis*, 5th ed., 28 (1927). American Public Health Association, Inc., New York, N. Y.
100. *Münch. med. Wochschr.*, **53**, 540 (1906).
101. *J. Infectious Diseases*, **7**, 632 (1910).
102. *J. Dairy Sci.*, **16**, 59 (1933).
103. *J. Dairy Sci.*, **36**, 1267 (1953).
104. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 303 (1911).
105. *Wis. Agr. Expt. Sta., 24th Ann. Rept.*, 231 (1907).
106. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 117 (1909).
107. *J. Dairy Sci.*, **33**, 676 (1950).
108. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 380 (1914).
109. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 290 (1952).
110. *J. Dairy Sci.*, **16**, 79 (1933).
111. *J. Dairy Sci.*, **32**, 649 (1949).
112. *J. Milk Technol.*, **5**, 323 (1942).
113. *J. Dairy Sci.*, **27**, 215 (1944).
114. *Vet. Record*, **63**, 32 (1951).
115. *U. S. Dept. Agr. Tech. Bull.* 992 (1949).
116. *Wis. Agr. Expt. Sta. Research Bull.* 6 (1909).
117. *U. S. Dept. Agr. Circ.* 400 (1936).
118. *U. S. Dept. Agr. Circ.* 672 (1943).
119. *J. Milk Technol.*, **3**, 75 (1940).
120. *Vt. Agr. Expt. Sta. Bull.* 547 (1948).
121. *Can. Public Health J.*, **30**, 44 (1939).
122. *Cornell Vet.*, **31**, 47 (1941).
123. *J. Dairy Sci.*, **26**, 295 (1943).
124. *J. Dairy Sci.*, **21**, 739 (1938).
125. *J. Dairy Sci.*, **17**, 257 (1934).
126. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 211 (1936).
127. *J. Dairy Sci.*, **19**, 395 (1936).
128. *J. Comp. Pathol. Therap.*, **59**, 81 (1949).
129. *Proc. Soc. Appl. Bacteriol.*, No. 1, **29** (1946).
130. *Mo. Agr. Expt. Sta. Research Bull.* 454 (1950).

3 Milk Fermentations

GENERAL CONSIDERATIONS

A milk fermentation is a process by which a change is produced in milk through the activity of micro-organisms. As milk normally leaves the udder, it is very satisfactory for the growth of many species and, accordingly, undergoes a series of changes; the processes producing these changes constitute the milk fermentations. Many of the fermentations that occur in milk also occur in various milk derivatives.

Normal and Abnormal Fermentations. The common change occurring in raw milk is development of lactic acid with the resulting coagulation of the casein. The process involved is designated the lactic acid fermentation, or the souring of milk. Because of the regularity with which it occurs, it is called the normal fermentation of milk. The processes causing other changes, such as sweet curdling, development of ropiness, etc., constitute the abnormal milk fermentations.

Ordinarily, a milk fermentation can be caused by more than one species and sometimes by several species. However, the most convenient basis for study of the changes occurring in milk as a result of action of organisms is a division into the different types of changes, rather than an attempt to classify all the causative species. The principal reason for this is that the change taking place is the important thing under practical conditions and, frequently, information with ref-

erence to a fermentation can be given without knowing the specific organism involved so that valuable time is saved in correcting the difficulty. If a consideration of the changes occurring in milk were based on a classification of the organisms responsible, the first step in dealing with any abnormal condition would be the isolation and identification of the causative species and this is time-consuming. An objection to the plan of studying the different fermentations as units is that organisms not at all related often are thrown together because they happen to have one common character, such as development of gas or ropiness in milk; for example, a discussion of the gassy fermentation involves a consideration of widely differing organisms.

Mixed Fermentations. It often happens that two or more fermentations occur in milk at the same time; production of acid may be accompanied by gas formation or a color change, development of ropiness may occur along with a change in flavor, or there may be other groupings. Often these combinations of fermentations are due to the activity of more than one species, but some organisms in pure culture produce two or even more changes in milk. The *Escherichia-Aerobacter* group produces gas, off flavors, and acid, and certain cultures also produce ropiness so that this group should be considered under at least four fermentations. Although this is an extreme example, there are many species that produce more than one type of change.

Variations in Organisms. Organisms that apparently belong to the same species may differ in a character that is important from the standpoint of milk fermentations. The best illustrations of this are in connection with ropiness; some cultures of *Lactobacillus bulgaricus* produce a ropy condition, whereas others do not, and this same variation occurs with a number of other species, including *S. lactis*. Variations in flavor production also occur among organisms belonging to the same species.

Associative Action. Associative action is the combined action of two or more organisms in producing a change which cannot be produced by one of them alone. Action of the organisms growing together often can be traced to the influence of the products of one on rate of growth or products of the other, but in some instances no such simple explanation is justified.

The effect of organisms growing in combination is particularly important in dairy products and can influence amount of acid produced, development of ropiness, color production, flavor development, etc. It is probable that associative action often is involved in the changes occurring in milk and cream on extended holding, especially changes in flavor. Failure to isolate an organism capable of producing a cer-

tain fermentation in a dairy product sometimes is due to the change being the result of action of organisms in combination. When a pronounced change in milk is due to associative action, the species involved often are present in considerable numbers so that isolations are not difficult.

LACTIC ACID FERMENTATION

In raw milk held at favorable temperatures, various species of bacteria produce lactic acid from lactose. Commonly, enough acid is formed to precipitate the casein and the liquid becomes a semisolid mass.

Frequency of the Fermentation. Lactic acid fermentation occurs in raw milk in all parts of the world, its rate being determined primarily by the holding temperature. In certain areas souring may be markedly delayed; new dairy districts may not have the surroundings thoroughly seeded with lactic acid organisms, and some older districts may have a climate that does not encourage the organisms in their natural habitats.[1] Lactic acid is produced through bacterial action in various materials, such as silage and sauerkraut, in addition to dairy products.

Historical. Although living organisms were observed in sour milk early in the eighteenth century, it was not until 1837 [2] that they were suggested as the cause of the souring. The idea of the causal relationship of organisms to this change found few advocates until Pasteur,[3] in 1857, definitely showed the existence of lactic acid-producing organisms. In 1878, Lister [4] succeeded in isolating one of these forms and thus provided incontrovertible proof. Before the importance of organisms in bringing about various decompositions was recognized, the change from lactose to lactic acid was thought by certain investigators to result from something in the casein.

Lactic Acid. Lactic acid was discovered in sour milk by Scheele, in 1780, but it was not until many years later that its constitution was established as $CH_3 \cdot CHOH \cdot COOH$ (α-hydroxypropionic acid). It is nonvolatile and miscible with water, alcohol, or ether in all proportions. Commercial lactic acid is a colorless, somewhat viscous liquid having a strongly acid taste and containing water. By distilling the aqueous solution under low pressure (1 mm), lactic acid can be purified and obtained as a crystalline solid melting at 64.4 F; when heated at ordinary pressures, it is converted partially to the anhydride before dehydration is complete. Pure lactic acid is odorless.

Transformation of Lactose to Lactic Acid. The transformation of lactose to lactic acid can be generalized as follows:

$$C_{12}H_{22}O_{11} \text{ (lactose)} + H_2O = 4C_3H_6O_3 \text{ (lactic acid)}$$

The first step is the hydrolysis of lactose to glucose and galactose. At least in the dissimilation of glucose, the sequence appears to be glycericaldehyde phosphate, phosphoglyceric acid, pyruvic acid, and lactic acid.

The transformation of lactose to lactic acid is not quantitative, and the fermentation yields other products, the so-called secondary products, which may include various acids, aldehydes, alcohols, gases, etc. The nature and quantity of the secondary products vary greatly with the different organisms.

Optical Isomers of Lactic Acid. Lactic acid contains an asymmetric alpha carbon atom and exists in two configurations (D and L) and in two optically isomeric forms [dextrorotatory (+) and levorotatory (−)]. Some organisms produce D(+) acid only, others produce D(−) acid only, and still others produce D(+) and D(−); of the organisms that produce both optical isomers, some form them in equal amounts (racemic mixture), whereas others do not. The optical isomer produced by an organism in milk appears to be constant for a given organism regardless of the holding temperature, although with organisms producing both D(+) and D(−) acid, the one produced in the smaller amount may be absent under conditions unfavorable for growth. In naturally contaminated milk, the optical rotation is influenced by the holding temperature because of its effect on the relative growth rates of the various organisms.

Acidity of Fresh Milk. The acidity of fresh milk, calculated as lactic acid, varies rather widely. Most values for herd milk fall between 0.14 and 0.19%; milk from individual cows shows greater variation. Little, if any, lactic acid is present in fresh milk, the acidity being due to carbon dioxide, casein, albumin, phosphates, and citrates (see p. 31).

Changes in Acidity of Milk. When ordinary raw milk is allowed to stand at room temperature, the acidity soon begins to increase. The acidity at which milk tastes sour varies with different persons and also with the initial acidity, a definite increase in acid being more important than the actual acidity. With most people, milk having an acidity of 0.25% tastes sour and, with many, milk having definitely lower acidities tastes acid. Persons accustomed to judging milk flavors frequently detect an acid flavor at acidities below 0.20%, especially when the milk had a low initial acidity. Acid is first detectable

by taste at a *p*H of about 6.0.[5] Certain flavors associated with development of acid may be noted when the acidity is increased only very slightly.

As the acid in milk continues to increase, the casein precipitates at an acidity varying with different samples; usually, the precipitation occurs between 0.50 and 0.65%. Van Slyke and Baker [6] found that the casein begins to coagulate when the *p*H reaches 4.64 to 4.78. On boiling, milk commonly curdles at an acidity varying from 0.30 to 0.45%.

With many lactic acid-producing bacteria, the acidity continues to rise after coagulation of the casein until the accumulation of acid prevents further growth. The maximum amount of acid developed in milk varies with different species, and even with different cultures of the same species. The lactose, which averages about 5.1% in milk, is very rarely, if ever, the limiting factor in acid production, and under ordinary conditions there still is considerable lactose remaining when development of acid has stopped. Van Slyke and Bosworth [7] found that in fresh milk inoculated with two common organisms, *S. lactis* and *A. aerogenes*, and allowed to stand at room temperature for 60 hr, 22% of the lactose disappeared and 88.5% of it was converted to lactic acid.

The spontaneous souring of milk involves chemical changes other than formation of lactic acid. Van Slyke and Hart [8] pointed out that the acid produced combines with the bases of some of the inorganic salts and then with the calcium that is in combination with the casein, which results in formation of a precipitate of free casein. As more acid is produced, the free casein unites with it, forming a casein salt of the acid, and this constitutes the curd noted in sour milk. The citric acid present in milk commonly disappears during the souring.[9]

Preservative Action of Lactic Acid. The lactic acid produced in milk prevents growth of many species of bacteria, especially putrefactive types, because of their lack of acid tolerance. Thus lactic acid acts as a preservative. Rapid decomposition of sour milk does not occur until the acidity has been decreased. Under the usual conditions this is brought about by various micro-organisms, particularly molds, which use part of the acid and neutralize part with products of their metabolism. In raw milk allowed to stand at room temperature, development of acid, decrease in this acid largely through growth of molds, and then rapid decomposition constitute a fermentation cycle that occurs with great regularity.

With sour milk and cream, objectionable flavors other than those of a putrefactive nature develop on standing so that the holding pe-

riod is limited under ordinary conditions. This change is due largely to activity of micro-organisms, but chemical action probably also plays a part in certain instances.

Lactic Acid Organisms. At the time Lister made the first isolation of a lactic acid organism, many people still doubted the causal relationship of micro-organisms to fermentations. With development of a technic satisfactory for the study of bacteria, the action of different species in changing a portion of the lactose in milk to lactic acid definitely was established. These organisms vary widely in their general characters. Some of them form relatively large amounts of lactic acid, and its production is the conspicuous change when growth occurs in dairy products; with others, production of lactic acid is not an outstanding character, and the organisms are of primary importance because of some other effect. If an organism is to be of significance in the production of lactic acid in milk, it must be capable of fermenting lactose. Species closely related to the typical lactic acid organisms are of little importance in milk because of their failure to ferment lactose, although they ferment some sugars readily.

The bacteria which are characteristically lactic acid-producing organisms in that they attack lactose actively and produce lactic acid in relatively large amounts from the lactose fermented are (1) *Streptococcus lactis* group and closely related forms, (2) *Streptococcus liquefaciens* group, (3) certain species of the genus *Lactobacillus,* and (4) certain species of the genus *Bacillus.* The organisms with which production of lactic acid is less important include various species. *Escherichia-Aerobacter* organisms form some lactic acid from lactose, but they also produce gases and other compounds that ordinarily are of more importance. *Streptococcus paracitrovorus* produces lactic acid from lactose, but the amount is small and the organism is of interest primarily because of its ability to ferment citric acid with formation of compounds important in the desirable flavors of certain dairy products.

Streptococcus lactis Group and Related Organisms

Certain of the streptococci play a striking role in the development of acid in dairy products. The more important of these constitute the *S. lactis* group, but there are closely related forms that also are of significance. At favorable temperatures the organisms produce lactic acid in milk rapidly, or fairly rapidly, without digestion or formation of gas; litmus, when present, commonly is reduced rather rapidly.

1. ***Streptococcus lactis* Group.** The organism isolated by Lister (1878) [4] and named *Bacterium lactis* by him belongs to what is now known as the *S. lactis* group. Leichmann (1896) [10] proposed the name *Bacterium lactis acidi* for what undoubtedly was the same organism, and Kruse (1903) [11] suggested *Streptococcus lacticus* because he considered the organism a *Streptococcus.* Various other designations also have been proposed. *S. lactis* is the name now commonly used, since it retains the original species name and replaces the original generic name with one commonly employed in systems of nomenclature.

Variations in Group. *S. lactis* refers to a group of organisms, the members of which show variations in the flavor produced, consistency of the fermented milk, rate of reduction of litmus, rate of acid production, etc. These differences are of significance because of the frequency with which the organisms develop in dairy products. On the basis of variations commonly encountered, Hammer and Baker [12] suggested the following varieties of *S. lactis.*

S. lactis var. *maltigenes* is characterized by the production of a malty, or caramel, flavor in milk, cream, etc. There are variations among strains in intensity of the flavor. The organism can produce a malty condition with no significant increase in acidity, and sweet milk or cream sometimes is distinctly malty, although sour products are involved more commonly. Frequently, the organism is present in such small numbers that it does not influence the flavor. Production of a malty flavor is a stable character that persists through many transfers of a culture; it occurs in media other than milk and cream when the organism grows well in them. *S. lactis* var. *maltigenes* is isolated readily from various materials showing a malty flavor unless they have been heated and, occasionally, is encountered in normal products. *S. lactis* organisms that produce a malty flavor in milk have been encountered by various investigators.[13,14] Apparently, they enter milk from various sources and may be present in the cows' udders.[15]

Organisms other than *S. lactis* var. *maltigenes* also have been reported as producing a malty flavor in dairy products. Apparently, they belong to various genera.[16] Tracy and Ramsey [17] found a malty flavor could be caused by a *Micrococcus;* when an organism of the *B. subtilis* group also was present, the flavor was more pronounced, whereas acid-forming bacteria retarded its development.

A malty flavor produced in milk has been ascribed to acetaldehyde.[18] More recently, Jackson and Morgan [19] reported that the flavor produced by *S. lactis* var. *maltigenes* is due principally to 3-methylbutanal;

as little as 0.5 ppm of the compound added to milk simulates the characteristic aroma.

S. lactis var. *hollandicus* produces a ropy, or slimy, condition in milk, cream, etc. There are variations among strains in extent of the ropiness, some producing only slight ropiness, others producing a conspicuous condition. The ropy character is not stable; a culture often varies from time to time in extent of the ropiness produced and may lose entirely the ability to cause ropiness, especially when grown at a relatively high temperature. Hammer [20] obtained nonropy cultures in small numbers from ropy ones by plating and picking colonies and

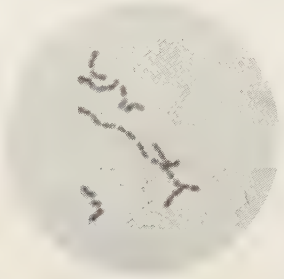


Figure 4. *Streptococcus lactis* in pairs and chains.

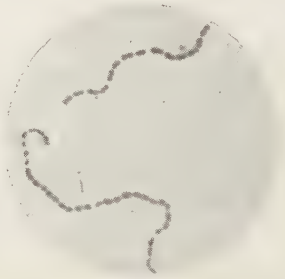


Figure 5. *Streptococcus lactis* in long chains.

also obtained a few ropy cultures from nonropy ones in the same manner. The microscopic pictures accompanying ropiness vary greatly. Some ropy cultures show chains of cells, others show clumps, and still others show great numbers of cells in pairs. *S. lactis* var. *hollandicus* often can be isolated from ropy lactic culture or sour cream but is unusual in nonropy materials.

S. lactis var. *anoxyphilus* differs from the typical *S. lactis* in that it reduces litmus and comparable indicators slowly. Instead of rather completely reducing litmus milk before coagulation begins, the milk is partially, or completely, coagulated before reduction. Finally, reduction is practically complete and the culture has the usual appearance of an *S. lactis* culture so that unless observations are made at the proper time the slow reduction is not detected. The character is a stable one and persists in a culture over extended periods. It does not appear to be of practical significance. *S. lactis* var. *anoxyphilus* occasionally is isolated when dairy products are plated and colonies picked.

S. lactis var. *tardus* coagulates milk more slowly than the other varieties of *S. lactis*. There is considerable variation in the coagulation

rates of different strains. In general, the delayed coagulation is due to relatively slow multiplication of the cells and the delay is greater at 70 F than at 98.6 F. By plating and picking colonies, cultures of S. *lactis* var. *tardus* often can be obtained in small numbers from cultures that coagulate rapidly. Harriman and Hammer [21] found that this occurred regardless of how many times the rapid cultures were purified by plating. The slow coagulation rate of S. *lactis* var. *tardus* is a stable character, and this variety does not yield rapidly coagulating cultures when it is plated. The organism often is isolated when sour milk or cream is plated and colonies picked.

Undoubtedly, there are other definite varieties of S. *lactis*. For example, Krishnaswamy and Babel [22] suggested that S. *lactis* cultures which produce appreciable amounts of biacetyl be recognized as a variety and designated S. *lactis* var. *aromaticus*. A culture obtained from a commercial lactic culture by plating and further purified contained both appreciable and low biacetyl-producing strains.

Morphology. S. *lactis* is a practically spherical form that may be slightly longer than broad, especially just before division. Size of the cells in different cultures varies and may be influenced by age of the culture, food materials present, growth temperature, etc.; the diameter usually falls between 0.5 and 1.0 μ. When grown in milk, the cells ordinarily are arranged in pairs or short chains although cultures showing long chains occasionally are encountered; rarely, pairs of cells are grouped into clumps. In liquid media other than milk, chains may be formed, but on solid media they are unusual. The organism is gram positive, stains readily with ordinary stains, and is nonmotile. Capsules often are present although usually found on only a portion of the cells in a preparation.

Cultural Characteristics. S. *lactis* grows well in milk and certain milk derivatives. It develops fairly well on standard (for milk), tomato, or whey agar. Growth in bouillon is meager, unless some material that the organism is capable of fermenting is added. The most characteristic of the cultural reactions is that in litmus milk. Ordinarily, the first observable change is a reduction of the litmus which usually begins at the bottom and progresses toward the top; reduction of the litmus often is practically complete before coagulation is evident, although this is not the case with S. *lactis* var. *anoxyphilus*. Coagulation also commonly begins at the bottom and, when complete, it is usual to find the milk white, except for a narrow pink band at the surface. As the culture ages, this pink band gradually

increases in depth until the whole tube is pink. There is no digestion or gas formation.

Colonies on agar are small, white, smooth-edged, and nonviscous. Commonly, surface colonies are round and subsurface colonies ellipsoid.

Biochemical Characteristics. *S. lactis* ordinarily produces acid from glucose, galactose, fructose, lactose, and maltose; other common test materials may, or may not, be fermented. Lactic acid regularly is produced from the lactose in milk, and at favorable temperatures the rate is rapid; volatile acids also are formed in small amounts. The maximum acidity developed in milk by most cultures is between 0.7 and 1.0% although occasionally up to 1.25% is encountered. The percentage of the theoretical yield of lactic acid ($C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3$) produced by *S. lactis* varies from 88 to 98%. The lactic acid formed is D(+).

White and Sherman [23] found that under strictly aerobic conditions, obtained by vigorous and complete aeration, the percentage of lactic acid produced by *S. lactis* per unit of glucose fermented ranged from 11 to 63%; anaerobic controls regularly gave more than 95%. Bång [24] noted that the decomposition of sugar by *S. lactis* can take place both homo- and heterofermentatively, the latter occurring at lower temperatures (59 to 68 F). Apparently, the lower temperatures cause changes in the enzyme system so that the products of metabolism vary with temperature. Acetylmethylcarbinol appeared to be formed from sugar at low temperatures.

Protein Breakdown. Various investigators have found that when *S. lactis* is grown in milk there is an increase in the soluble nitrogen. The increase is greater when calcium carbonate is added to the milk and frequently distributed by shaking during the fermentation since this neutralizes some of the acid formed and permits more extended growth. The protein breakdown goes to the amino acid stage.

Morgan and Nelson [25] reported that microbiological assays for 10 amino acids performed on tungstic and lactic acid filtrates of skim milk cultures of 5 strains of *S. lactis* showed marked increases on extended incubation. van der Zant and Nelson [26] noted that *S. lactis* in milk caused a rapid increase in both soluble nitrogen and tyrosine and tryptophane during the first 24 hr, followed by smaller but gradual increases. Cell-free extracts [27] prepared from *S. lactis* grown in milk contained a heat-labile proteolytic enzyme system with optimum activity against milk, casein, and lactalbumin at *p*H values near neutrality. With paper chromatography [28] the protein-free fraction of

skim milk incubated with *S. lactis* for 0, 24, and 96 hr showed the presence of 3 peptides at 0 hr, 5 at 24 hr, and 6 after 96 hr. Cell-free extracts [29] prepared from *S. lactis* grown in milk contained several peptidases which had their optimum activity at *p*H values from 7 to 8.5.

Growth Conditions. *S. lactis* grows over a wide temperature range, with different cultures showing somewhat different temperature extremes. Many cultures are most active between 86 and 104 F although development also is relatively rapid at 70 F. Growth is comparatively slow at 50 F, commonly does not occur at 113 F, and a few cultures also fail to grow at 98.6 F. In general, the maximum acidity produced is somewhat higher at 70 F than at 98.6 F, although the period required is longer. Acid production is somewhat more rapid with a restricted than with an abundant air supply. *S. lactis* is destroyed easily by heat. Milk cultures die rather quickly because of the destructive action of the acid formed.

Fermenting Capacity of *S. lactis* Cells. The lactic acid produced per individual cell per hour in milk by *S. lactis* was found by Baker *et al.*,[30] using a microscopic count, to lie between 5×10^{-10} and 10×10^{-10} mg for a vigorous culture; the rate tended to decrease as the curdling point was approached. Considerable variation occurred in the rate of acid production with different cultures. There was a tendency for the acid contained, whether measured as titratable acidity or *p*H, to increase geometrically until coagulation.

At one time growth and acid production by *S. lactis* were considered to be more or less independent. It appears, however, that there is acid developed during the early growth period but that it is difficult to detect because it is so little. No relationship exists between the acidity and the number of cells of *S. lactis* in milk in which the organism is growing because, after the acidity has reached a certain point, the cells begin to die, while the acid production still may continue slowly.

Acids Other Than Lactic. The distinct acid odor of most cultures of *S. lactis* suggests that acid other than lactic has been formed, and determinations on cultures show a definite but rather small amount of volatile acid. Evans [31] reported that cultures of *S. lactis* produced a fairly constant quantity of acetic acid in milk, equivalent to about 0.12 g per liter. Hammer and Sherwood [32] found that the volatile acid produced by *S. lactis* was made up of acetic with considerable amounts of propionic.

Associative Action Involving S. *lactis.* Various organisms common in dairy products influence the amount of acid produced in milk by *S. lactis.* Marshall and Farrand [33] found that 24 of 42 organisms isolated from milk accelerated the growth, 11 had no effect, and 7 retarded it. The acceleration occurred at different stages in the development of *S. lactis;* apparently, it was due to products formed by the associated organism, and these sometimes were stable to heat and sometimes not. When certain yeasts are grown in combination with *S. lactis* in milk, the period it retains its vitality is increased greatly.[34] Cox and Whitehead [35] reported that *B. subtilis* appeared to stimulate production of acid by *S. lactis,* whereas *E. coli* caused a decrease in some experiments and an increase in others.

S. lactis inhibits growth of various bacteria in milk, presumably through production of acid. It also may inhibit an organism which produces considerable acid, since Rogers [36] noted that, in mixtures of *S. lactis* and *L. bulgaricus* containing a predominance of *S. lactis,* the *Lactobacillus* was suppressed by a specific substance diffusible through a collodion membrane; it was not destroyed by boiling. The same substance apparently also limits growth of *S. lactis* itself.[37] Baribo and Foster [38] found that 9 strains of *S. lactis* and *Streptococcus cremoris* and 3 lactic cultures produced a heat-stable substance that inhibited growth of *Lactobacillus casei;* it also inhibited certain strains of lactic streptococci but to a lesser extent. The lactic streptococci varied in their ability to produce the inhibitor.

Importance of S. *lactis.* *S. lactis* is the species mainly responsible for the souring of milk and cream under practical conditions. In this way it causes great economic losses since sour milk and cream cannot be used for various purposes for which the sweet products are employed.

S. lactis plays a necessary part in the preparation of certain dairy products and, therefore, is beneficial as well as harmful. It is important in preparation of lactic culture, in ripening of cream for butter, in manufacture and ripening of many cheeses, and in making various fermented milk products; its principal function in all these cases is the formation of acid.

Distribution and Sources of S. *lactis.* At the time milk leaves the stable it practically always contains *S. lactis,* but the percentage of the total flora made up of the organism is small. Ordinarily, it does not come from the interior of the udder.

Probably the most common source of *S. lactis* under practical farm conditions is the utensils, and often it can be obtained from the coat

of the cow, silage, and various grains. Esten [39] found it in the feces of 11 of 25 cows and obtained it from the mouths of 17 of 21 cows; mangers and materials within reach of a cow's mouth were well seeded with it. McGuire [40] found the organism normal to cow feces. Stark and Sherman [41] repeatedly isolated *S. lactis* from certain plants but not from all plants examined and did not find it in mouths or throats of cows, in bovine or human feces, or in soil. They suggested that plants may represent the natural habitat of the organism.

Isolation of *S. lactis.* *S. lactis* is isolated easily by allowing raw milk or cream to sour at room temperature, plating on a suitable agar, incubating the plates at 70 F (21.1 C), and then picking colonies into litmus milk. In recently soured milk, *S. lactis* ordinarily makes up 90 to 99% of the flora so that most of the colonies picked will be *S. lactis*, even when no attempt at selection is made. Colonies of *S. lactis* are distinguished easily from those of many, but not all, of the organisms common in milk. When an attempt is made to isolate *S. lactis* from fresh milk by direct plating, difficulty frequently is encountered because of the small numbers in which it is present.

2. **Organisms Closely Related to *Streptococcus lactis.***

Streptococcus cremoris. Certain investigators consider *S. cremoris* a species distinct from *S. lactis* on the basis of a tendency to form long chains in milk, inability to produce ammonia from 4% peptone, and failure to grow in the presence of 4% sodium chloride, at *p*H 9.2, or at 104 F. None of these differences appear to be important from the standpoint of the usual growth of the organisms in milk.

S. cremoris sometimes is considered to be the typical acid producer in lactic cultures. Nichols and Hoyle [42] reported that of many strains from a wide range of lactic cultures none were *S. lactis.* With "wild" strains selected as suitable for lactic cultures on the basis of acid production, grouping as either *S. lactis* or *S. cremoris* was difficult since many appeared to be intermediates. If the "wild" strains were divided into 2 species, only 2 could possibly be *S. cremoris,* although some exhibited one, or more, of its characteristics. With the same latitude in interpretation, 65% were *S. lactis* and the remainder probably variants of it. Sherman [43] has emphasized the close relationship of the 2 organisms and indicated that the hardier *S. lactis* may be the more desirable in lactic culture for cheese.

Cultures of lactic streptococci from various sources have been used in developing lactic cultures. Such organisms may be in pairs or short chains when first inoculated into milk and then be in longer chains after many transfers. In some excellent lactic cultures, even

after many transfers, the organisms still are largely in pairs. In a lactic culture the organisms have been through many transfers under favorable growth conditions and probably are more sensitive to unfavorable conditions than organisms growing on utensils, in manure, etc.

Streptococcus thermophilus. *S. thermophilus* differs from *S. lactis* primarily in its temperature relationships. It grows well at 113 F and at 98.6 F, but growth is slow at 70 F and does not occur at 50 F. The organism also is capable of surviving comparatively high heat exposures. Because of its temperature relationships, *S. thermophilus* is the important lactic *Streptococcus* in milk and other dairy products that have been subjected to relatively high heat. Often it is isolated easily from pasteurized milk, particularly if the milk is incubated at temperatures favorable for its growth.

S. thermophilus does not reduce the litmus in litmus milk as completely as a typical culture of *S. lactis*, and the top one-third, or more, of a tube may show a pink color, even when reduction is at the maximum. It produces D(+) lactic acid.

Additional Organisms. Various organisms essentially like *S. lactis* sometimes have been considered as distinct species on the basis of fermentation tests and other minor variations. This practice results in a multiplicity of species that is very confusing. Hunter [44] studied a lactic *Streptococcus* which grew on agar containing a certain peptone only under conditions of semianaerobiosis. However, it grew on the surface if (1) the peptone was replaced by tryptone, (2) the lactose in the medium was replaced by glucose, or (3) the *p*H was reduced.

Streptococcus liquefaciens Group

Streptococcus liquefaciens rapidly coagulates milk and then actively proteolyzes it. The coagulation occurs at a low acidity and is due to a rennin-like enzyme. With 15 cultures studied by Long and Hammer [45] the titratable acidities at coagulation ranged from 0.25 to 0.29% and averaged 0.27; the *p*H values varied from 6.02 to 5.85 and averaged 5.90. Following coagulation the acidity continues to increase until it is essentially the same as that produced by *S. lactis*. Proteolysis is evident soon after coagulation. It increases and the mass of curd becomes smaller and smaller as it contracts from the wall of the container and the surface of the liquid. Milk that shows definite proteolysis has a bitter flavor due to protein decomposition products.

When litmus is present in the milk, coagulation commonly is preceded by reduction of the litmus that is complete except at the surface.

With some cultures reduction is delayed and is not complete until after coagulation; from this standpoint, the slow reducing cultures are comparable to S. *lactis* var. *anoxyphilus*. Before proteolysis is evident, litmus milk cultures of S. *liquefaciens* appear essentially the same as cultures of S. *lactis*.

General Characters. S. *liquefaciens* is a typical *Streptococcus*. In milk the cells are arranged singly, in pairs, and in short chains. Except for proteolysis of milk and liquefaction of gelatin, the general cultural characters are essentially the same as those of S. *lactis*.

The lactic acid produced is D(+). Acetylmethylcarbinol regularly is produced in milk, and some cultures form relatively large amounts; the yield commonly is much greater at 70 F than at 98.6 F. With certain cultures production of acetylmethylcarbinol is increased by addition of citric acid to the milk.

S. *liquefaciens* grows at 50 and 113 F; growth is much more rapid at 98.6 F than at 50 F. Cultures survive 143 F for 20 min; most of them survive for 50 min, but all of them are destroyed at 150 F in 40 min.[45] The organism survives in cultures much longer than S. *lactis*.

Distribution and Importance. S. *liquefaciens* is widely distributed in dairy products but ordinarily is not present in large numbers. It has been isolated most regularly from cheddar cheese.[45] The organism is of little importance in production of acid in raw milk and cream, but in certain instances it apparently has been responsible for bitterness in pasteurized products.

Isolation. The proteolytic property of S. *liquefaciens* is an aid in its isolation. Materials can be plated on agar to which sterile milk has been added and, after incubation, actively proteolytic colonies that suggest streptococci can be picked into litmus milk and studied. When the organism is present only in small numbers, there is an advantage in using heavily seeded plates so that a relatively large amount of material can be examined; with incubation at 98.6 F (37 C) for 16 to 24 hr S. *liquefaciens* develops well, but other species ordinarily do not grow to such an extent that they interfere seriously with picking of the desired colonies. In attempting to pick S. *liquefaciens* from plates, proteolytic micrococci are responsible for most of the failures.

Species of the Genus *Lactobacillus*

The genus *Lactobacillus* includes organisms that fall into two natural groups, the heterofermentative species, which produce a variety of compounds from the sugar fermented, and the homofermentative

species, which produce principally lactic acid from it. The homofermentative organisms that attack lactose are of special significance in dairy products because of their wide distribution and activity under certain conditions. Their general action on milk is much like that of S. *lactis*, and the striking difference between the two groups is in morphology. They sometimes are referred to as the rod-shaped lactic acid organisms. Certain of the organisms are acid tolerant and are capable of growing in milk and other dairy products after growth of S. *lactis* has been stopped by the accumulated acid.

Presence of Lactobacilli in Dairy Products. Lactobacilli commonly are present in dairy products. Following investigations of the organ-

Figure 6. *Lactobacillus acidophilus.*

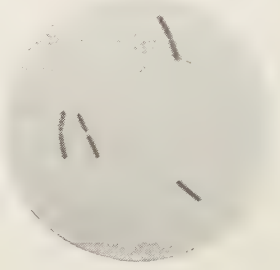


Figure 7. *Lactobacillus bulgaricus.*

isms in Europe, their wide distribution in milk, butter, and cheese in the United States was reported; [46] they also were found in feces of various animals, sour and aromatic foods (dill pickles, olives, and sauerkraut), cattle feed, normal saliva and gastric juice, fermented milk, market milk, and soil.[47] Lactobacilli play little part in the beginning acid development in milk and cream because of rapid growth of S. *lactis*, but, if holding is extended, they become active when other species have produced their maximum acidities. In certain areas producing sour cream for butter, the cream occasionally shows excessive acidities during the warm months, owing to the action of lactobacilli. Whey in vats or returned to the farms has been found to contain many of the organisms.[48] They commonly are present in ripened cheeses, often in large numbers.

The homofermentative lactobacilli that attack lactose are well adapted to growth in dairy products generally. They are favored greatly by the lactose, and their acid tolerance permits development under conditions which inhibit many bacteria.

Species Important in Dairy Products. Apparently, several species of homofermentative lactobacilli are important in dairy products. Their classification presents difficulties and what appears to be the same organism may be referred to by more than one name.

The organism now known as *L. casei* apparently was first studied by von Freudenreich [49,50] in investigations on the ripening of swiss cheese. It quite regularly is present in milk in the United States and at least certain other countries. Sherman and Stark [51] noted that *L. casei* generally is present in milk in considerably larger numbers than either *L. acidophilus* or *L. bulgaricus.* With grade A milk, only 12% of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers as great as 1 per ml, whereas 71% contained *L. casei* in such numbers; with ordinary milk, only 2.3% of the samples contained *L. acidophilus* or *L. bulgaricus* in numbers exceeding 10 per ml, whereas 94% contained *L. casei* in such numbers.

L. acidophilus first was isolated from the fecal material of infants by Moro [52] and now is recognized as more or less regularly present in the normal intestinal tract of man and other warm-blooded animals. Cultures of the organism are used in attempts to control the intestinal flora; it appears to colonize better than other lactobacilli.

L. bulgaricus originally was isolated by Grigoroff [53] from fermented milk. It received considerable attention as a result of the recommendation of Metchnikoff that it be used to control intestinal putrefaction which this investigator believed was an important factor in limiting the length of life in man. The suggestion was based on the theory that since putrefactive organisms are sensitive to acid their development in the intestinal tract can be controlled by organisms producing considerable acid. *L. bulgaricus* was selected by Metchnikoff because it produces a comparatively high acidity in milk and also because of the presence of the organism in the sour milk consumed in large quantities by certain races noted for their long life. However, extensive trials indicate that *L. bulgaricus* does not establish itself in the intestinal tract, even when consumed in large numbers.

Lactobacillus helveticus first was isolated from emmenthaler cheese and since has been isolated from other cheeses, fermented milks, etc. *Lactobacillus plantarum* has been isolated from milk, butter, and cheese, as well as from certain nondairy products.

General Description of Homofermentative Lactobacilli. Morphology. The organisms are definite rods that vary a great deal in size, with *L. bulgaricus* tending to be larger than the others. The cells may occur singly or in chains; some *L. bulgaricus* cultures show unusually

long chains. The organisms stain readily and are gram positive although some negative cells often are noted, especially in old cultures. Occasionally, stained preparations show distinct granules.

Cultural Characters. The organisms grow especially well in milk. In litmus milk there ordinarily is coagulation and reduction of the litmus, except for a pink band at the top. Gas is not produced. Growth is definite, but slight, in bouillons containing various fermentable materials. Whey or tomato agar has been used extensively for the organisms. In general, colonies are small and somewhat variable in type with the edges either smooth or filamentous; some *L. bulgaricus* cultures give a very filamentous edge. Various special agars [54,55] have been developed for the lactobacilli in attempts to improve colony development.

Biochemical Features. Acid is produced from a number of materials, but there is no great regularity in those fermented. In milk, acid develops readily at favorable temperatures. The final acidity varies a great deal with some cultures giving only slight coagulation in milk and others producing more than 4% acid; *L. bulgaricus* yields especially large amounts. *L. casei* produces D(+) lactic acid, whereas *L. acidophilus, L. bulgaricus, L. helveticus,* and *L. plantarum* produce racemic lactic acid. The percentage of the theoretical yield of lactic acid is high, up to approximately 98 to 99%, and in accordance with this the cultures are lacking in odor. Proteolysis of the casein usually can be detected by chemical examination although it is not suggested by appearance of the milk cultures.

Growth Conditions. A comparatively high temperature, such as 90 to 100 F, greatly favors growth. *L. acidophilus* does not grow at 68 F or at 118.4 F and above.[56] *L. bulgaricus* does not grow at 68 F; the maximum growth temperature of fresh isolates may be 122 F or above, but for older cultures it is generally between 113 and 122 F. *L. casei* and *L. plantarum* grow at 59 F but not at 113 F. *L. helveticus* grows at 59 and 118.4 F. The organisms are microaerophilic.

Differentiation of Homofermentative Lactobacilli. The homofermentative lactobacilli that ferment lactose and are important in dairy products are differentiated only with difficulty. Various tests have been suggested for such differentiation, but most of them show irregularities when applied to a series of cultures. Wheater [56] found that *L. acidophilus* fermented amygdalin, cellobiose, salicin, and sucrose whereas *L. bulgaricus* did not. Also, *L. acidophilus* grew in 2% sodium chloride or bile salt whereas *L. bulgaricus* did not. *L. helveticus* fermented inositol, sorbose, glycerol, and rhamnose but not melibiose

or raffinose, failed to grow in 4% bile salt, and gave a positive Voges-Proskauer reaction. *L. casei* did not ferment these compounds nor tolerate 4% bile salt; it rarely gave a positive Voges-Proskauer test. *L. plantarum* always fermented melibiose, usually raffinose, and sometimes rhamnose but did not ferment inositol, sorbose, or glycerol. It tolerated 4% bile salt and gave a negative Voges-Proskauer test.

Importance of Lactobacilli in Dairy Products. Development of high acidity in cream intended for churning is undesirable because of the influence on the flavor of the butter. While the effect may be decreased by partial neutralization, it cannot be controlled entirely. In ripening of certain cheeses, lactobacilli play an important role since they are one of the factors involved in the protein decomposition. Lactobacilli also are desirable in certain fermented milk preparations.

Sources of the Lactobacilli. Trials at the Iowa Agricultural Experiment Station indicate that milk does not contain lactobacilli when it leaves the udder but that they are present by the time the milking is complete. The organisms regularly are found in the stable surroundings. Grains and other feeds contain them. They have been found in large numbers in silage [57,58,59] and are reported [58] to be present in considerable numbers on corn fodder so that corn silage always is amply seeded. The organisms are present in cow manure. Their presence in various feeds partly accounts for this, but multiplication in the intestinal tract also is a factor. Orcutt [60] found the organisms were present throughout the small intestine of young calves and were the predominating type in the fourth stomach.

Isolation of Lactobacilli. Lactobacilli are not readily isolated because of the difficulty with which they grow on media adapted to plating. In isolating from milk, a bottle should be filled, stoppered with a cork, and then held at a favorable temperature, such as 98.6 F, so that the desired organisms will increase in numbers. *S. lactis* and various other species multiply rapidly at first but, when they are restrained by the acid developed, the lactobacilli multiply. Microscopic examinations make it possible to follow the fermentation. When the lactobacilli are present in large numbers, the milk can be plated, the plates incubated at 98.6 F, and colonies picked into litmus milk. In some instances shake cultures yield better colony development than plates, owing presumably to the oxygen requirements of the organisms.

Acid tolerance of the lactobacilli makes it possible to check other species by means of added acid. One of the procedures used is to inoculate the material from which the isolation is to be made into bouillon containing a fermentable material, such as dextrose, and

enough acetic acid to give approximately an $N/10$ solution. Growth can be followed microscopically and, if the lactobacilli develop satisfactorily, plates can be poured. A series of transfers through the acid bouillon may yield essentially a pure culture of lactobacilli.

Species of the Genus *Bacillus*

The genus *Bacillus* includes species that differ conspicuously from many of the organisms in the group by producing a smooth curd and considerable lactic acid when grown in milk; if litmus is present, there commonly is pronounced reduction so that the cultures have the general appearance of *S. lactis*. Coagulation is due to a rennin-like enzyme, since it occurs at acidities such as 0.30 to 0.40%, but acid production continues until 0.50 to 0.90% is present. The organisms require comparatively high temperatures for active growth and are not found in significant numbers in milk held under the usual conditions. Occasionally, they are encountered in milk and certain of its derivatives when the holding temperatures are so high that the species usually active cannot grow, or when the method of treatment was such that only spore-forming organisms survived. Organisms of this general type have been of the greatest practical significance in evaporated milk, in which one of the types of spoilage is coagulation and acid development.

Bacillus coagulans[61] illustrates the lactic acid-producing organisms of the genus *Bacillus*. It grows well at 98.6 and 131 F, but poorly, if at all, at 68 F. The lactic acid produced in evaporated milk is D(+). Volatile acids also are formed and consist of acetic and propionic. Both the soluble and amino nitrogen are increased in milk. *Bacillus calidolactis*[62] is another organism of this general type. It grows rapidly at 131 F but does not grow at 98.6 F. In milk D(+) lactic acid is produced; the amount of volatile acid formed is small.

It appears that acid production in milk as a result of growth of organisms of the genus *Bacillus* is becoming more common. Usually, the milk involved is being held at relatively high temperatures in connection with the preparation of concentrated products.

GASSY FERMENTATION

Gas sometimes is produced in milk, cream, etc. Composition of the gas is variable, depending on the organism responsible, but carbon dioxide commonly is a prominent constituent. The organisms of most importance in producing gas in dairy products include (1) certain

nonspore-forming yeasts that ferment lactose, (2) the *Escherichia-Aerobacter* group, and (3) certain organisms of the genus *Clostridium.*

Importance of Gassy Fermentation. In dairy products definite gas formation occurs most often in materials containing some acid. It is common in sour cream held for considerable periods, especially during the warm months. In the extreme condition the covers are blown from the cans when the wires attaching them are clipped, and the

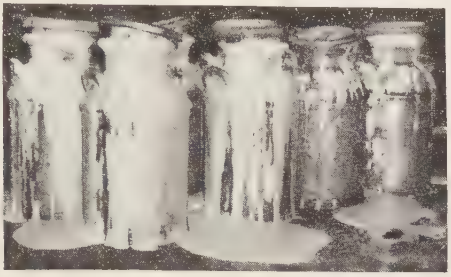


Figure 8. Yeasty cream. Courtesy R. V. Hussong.

cream foams or boils from the containers; if the covers do not fit properly, the cream may be forced out around the lids. When the fermentation is less extensive, gas production is evident on stirring the cream because of the tendency to foam. Gassy cream often is referred to as "yeasty" because of the pronounced yeasty odor, and investigations [63,64] have shown that the causative organisms are yeasts. Cream showing pronounced gas formation commonly is rejected by butter plants.

Development of gas sometimes occurs in milk being made into such products as cottage cheese and lactic culture; *Escherichia-Aerobacter* species are responsible in certain cases and yeasts in others. Various cheeses, especially cheddar, sometimes become gassy, with *Escherichia-Aerobacter* organisms commonly responsible. Whey standing in vats or cans occasionally undergoes a gassy fermentation which is most often due to yeasts; the change is favored by the relatively high temperatures at which whey commonly is allowed to stand.

Lactose-fermenting Yeasts

In the United States the yeasts of importance in the development of gas in dairy products include two species, *Candida pseudotropicalis* * (formerly *Torula cremoris*) and *Torulopsis sphaerica* * (formerly

* The names used follow the classification of Lodder and Kreger-Van Rij *The Yeasts. A Taxonomic Study* (1952). North Holland Publishing Co., Amsterdam.

Torula sphaerica). The organisms produce carbon dioxide and small amounts of ethyl alcohol from lactose; because of their acid tolerance, they can develop in materials in which the acid prevents growth of

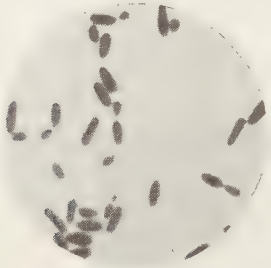


Figure 9. *Candida pseudotropicalis.*

Figure 10. *Torulopsis sphaerica.*

many organisms. The two species differ in shape and size of the cells and in growth temperatures. *C. pseudotropicalis* is ellipsoidal and grows better at 98.6 F than at lower temperatures whereas with *T. sphaerica* most of the cells are essentially spherical and there often is little or no growth at 98.6 F, the optimum temperature being somewhat lower. Both organisms produce vigorous gas evolution and a yeasty odor in litmus milk at favorable temperatures. Reddening of the litmus also occurs, and in old cultures there sometimes is coagulation, especially in the lower portions. The organisms grow well on agar containing lactose, and surface colonies are raised, smooth-edged, shiny, and chalk white; the plates have a yeasty odor.

Development of Yeasts in Cream. *C. pseudotropicalis* and *T. sphaerica* commonly are present in cream, either sweet or sour. In pure culture the organisms do not produce a typical foamy condition in cream. Without coagulation there is little tendency for the gas to be held and it readily escapes. However, when coagulation has occurred, the gas tends to be retained and the volume of the fermenting material is increased by the gas bubbles present. In either sweet or sour cream there commonly is a supersaturation with gas and, when this comes out of solution on jarring or agitation, it readily escapes from uncoagulated cream. With the coagulated product some is held and yields a foamy mass. Accordingly, typical foaminess in cream is due to associative action, the yeasts producing the gas and some other species causing the necessary coagulation.

Isolation of *C. pseudotropicalis* and *T. sphaerica*. One or both of the common lactose-fermenting yeasts often can be obtained from a sample of milk or cream by allowing it to stand at approximately 86 F until the odor and gas production suggest there has been a good growth of the desired organisms and then plating on some such medium as potato dextrose agar adjusted to *p*H 3.5.

Other Lactose-fermenting Yeasts. Additional lactose-fermenting yeasts have been described. Windisch [65] noted that the number known is small but that numerous yeasts can assimilate lactose.

Escherichia-Aerobacter Group

The *Escherichia-Aerobacter* organisms ferment lactose with the production of gas and acid. Gas production ordinarily is conspicuous in young milk cultures, and on being shaken they often develop a foam. Rate and extent of acid production vary greatly, some cultures causing coagulation rather rapidly, others failing to coagulate even after long holding. When coagulation occurs the curd is not especially gassy because the gas largely escapes before precipitation of the casein takes place. The organisms do not proteolyze the casein in milk although there sometimes is a collection of whey at the top of a culture that suggests this. Growth on agar plates appears quickly and the mature colonies are large.

Biochemical Characteristics. The gas produced by the *Escherichia-Aerobacter* organisms is primarily carbon dioxide and hydrogen, and the lactic acid is D(−). Lactose decomposition also yields other compounds, especially volatile acids.

Division of *Escherichia-Aerobacter* Group. There are two primary divisions of the organisms included in the *Escherichia-Aerobacter* group: (1) Those belonging to the genus *Escherichia*, of which *E. coli* is the type species, and (2) those belonging to the genus *Aerobacter*, of which *A. aerogenes* is the type species. An intermediate division also is recognized.

The genus *Escherichia* includes primarily the organisms coming from the intestinal tract of man and animals; the genus *Aerobacter* includes primarily those coming from soil and grains. Rogers *et al.*[66] called attention to the scarcity of the *Aerobacter* type in bovine feces and pointed out [67] that the *Escherichia-Aerobacter* organisms from grains do not agree with the characteristic flora of bovine feces. Malcolm [68] isolated 342 *Escherichia-Aerobacter* cultures from 114 specimens of bovine feces, and 96.4% were *E. coli*. By use of enrichment methods *Acrobacter* organisms often were isolated from fecal samples,

so it appears these organisms rather regularly are present but only in small numbers.

Presence of *Escherichia-Aerobacter* Organisms in Milk. Organisms of the *Escherichia-Aerobacter* group practically always are present in milk at the time it leaves the stable, although with very rigid methods of production relatively large volumes must be used to detect them. Ayers and Clemmer [69] found that milk, produced under the worst conditions normally encountered, rarely contained >2,000 per ml. Finkelstein [70] reported that when care was used in the production of milk the number of *Escherichia-Aerobacter* organisms ordinarily was small, averaging <100 per ml; with indifferent methods the number averaged 588.

With 21,569 samples of milk taken under fair conditions of cleanliness, Malcolm [71] found that 48.3% showed no *Escherichia-Aerobacter* organisms in 0.1 ml, 21.4% contained the organisms in 0.1 ml but not in 0.01 ml, 14% contained the organisms in 0.01 ml but not in 0.001 ml, and 16.3% contained the organisms in 0.001 ml. The proportion of positive samples was higher in summer and early autumn than in winter and spring, and there was a well-marked direct correlation between the proportion of samples containing the organisms in 0.1 ml and the minimum and maximum atmospheric temperatures. Yale and Eglinton [72] examined 882 samples of fresh milk obtained monthly from 85 producers and the *Escherichia-Aerobacter* counts were <1 per ml with 46%, <10 per ml with 83%, and <100 per ml with 95%; with 5% the counts ranged from 100 to 5,500 per ml.

Data of various investigators show that milk commonly contains both *Escherichia* and *Aerobacter* species. Levine [73] summarized data from various sources and found that, of 1,382 cultures isolated from milk, 43.1% belonged to the *Aerobacter* type. Hunter [74] reported that, of 590 cultures obtained from milk, 66.2% belonged to the *Escherichia* type, and of 169 cultures from bovine feces 95.2% also belonged there. Sackett and Gralak [75] found no correlation between the plate count and the coliform count of raw milk so that a low plate count did not necesarily mean a low coliform count and a high plate count did not indicate a high coliform count.

Sources of the Organisms in Milk. Organisms of the *Escherichia-Aerobacter* group occasionally have been found in milk at the time it leaves the udder, but this is unusual. They commonly get into milk through the general contamination with manure, soil, feeds of various types, flies, the milker, etc. They also may come from unsterile utensils, sometimes in large numbers.[69] Yale and Eglinton [72] reported the

Escherichia-Aerobacter counts on fresh cow manure ranged from 100,000 to 10,000,000 per g, and on dry cow manure they were as high as 90,000,000 per g. At two dairies using milking machines, *Escherichia-Aerobacter* organisms were recovered in large numbers, from the rubber parts at one dairy and from the milker pail at the other.

Growth of the Organisms in Milk. The *Escherichia-Aerobacter* organisms grow rapidly in milk when the temperature is suitable and multiplication may be the cause of excessive numbers. The growth temperatures vary with the species and also with the cultures grouped in a species. The organisms grow at 50 F, or even lower, and at 60 F growth may be quite rapid;[70] they sometimes grow in pasteurized milk held under refrigeration.

Hunter[74] investigated the effect of temperature on the *Escherichia-Aerobacter* organisms in milk by taking 59 samples from an average dairy and making counts at once and after holding one portion at 60 F and the other at 70 F for 24 hr. The fresh milk averaged 52 *Escherichia-Aerobacter* organisms per ml with variations from 1 to 1,000, that held at 60 F averaged 964 with variations from 0 to 10,000, and that held at 70 F averaged 60,762,000 with variations from 1,000 to 1,000,000,000. Many other investigators have shown that growth of *Escherichia-Aerobacter* organisms in dairy products often is rapid and that as a result definite defects are produced.

Importance in Dairy Products. The *Escherichia-Aerobacter* organisms always are undesirable in dairy products. In milk and cream, in addition to forming gas and acid, they produce objectionable flavors of various types; when such products are used in the manufacture of cheese or butter, the off flavors commonly are carried over and, unless the organisms are destroyed by pasteurization, development of the off flavors may continue. The *Aerobacter* and intermediate species appear to be similar in their general effects on flavor and produce a more serious and rapid deterioration than the *Escherichia* species.[76] Organisms of the group may play a part in the natural souring of milk during the initial stages although *S. lactis* is dominant later.[77]

In most of the studies on the *Escherichia-Aerobacter* organisms responsible for gas formation in dairy products, the identification has not been carried to a species basis. However, the studies along this line show that various species are involved.

Sarles and Hammer[78] reported an unusual instance in which a few consumers of a raw milk supply complained that the milk seemed to be full of gas. A bottle of milk returned by a consumer had a normal appearance, but on being shaken, or warmed, the milk foamed mark-

edly. An *Aerobacter* organism caused the defect. Claydon and Foltz [79] found an *Aerobacter* species the cause of a yeast-like defect in laboratory samples of milk and cream inoculated from dairy utensil sources. The defect produced in cream, particularly when the or-

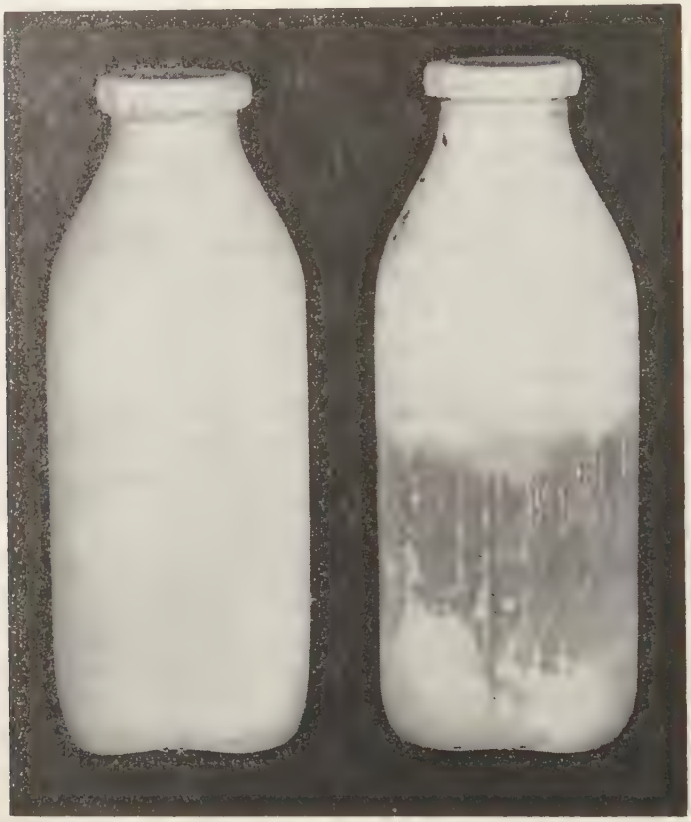


Figure 11. Associative action in the appearance of gas in milk; bottle at left shows action of the gas former alone, whereas bottle at right shows action of the gas former in combination with *Streptococcus lactis.*

ganism was associated with *S. lactis,* ranged from a mild "bread dough" odor to a definite yeast-like defect.

Isolation of *Escherichia-Aerobacter* Organisms from Dairy Products. Organisms of the *Escherichia-Aerobacter* group can be isolated directly from dairy products by plating on some differentiating medium, or an enrichment procedure, using bouillons of various types, may precede the plating. Detection of the organisms in dairy products

often is based on the development of gas in some selective medium (see p. 19).

Species of the Genus *Clostridium*

Certain of the spore-forming anaerobes of the genus *Clostridium* produce gas in milk or cream under favorable conditions, for example, when the acid-producing organisms are destroyed by heat and the material is held with a limited air supply. They are of little importance in raw milk or cream because of their inability to compete with the organisms normally present but may be of significance in certain products. Their action occasionally is noted in evaporated milk that was understerilized. The organisms are demonstrated easily in many lots of milk by heating, for example, at 176 F for 10 min, and then holding under essentially anaerobic conditions (p. 45).

ROPY OR SLIMY FERMENTATION

In the ropy or slimy fermentation of milk, cream, etc., growth of organisms results in such a change in consistency that the materials adhere and form threads, or viscous masses, when poured or dipped. There is great variation in extent of the ropiness; sometimes the change is so slight that it is detected only by careful examination, and in other cases the material can be drawn into threads a yard or more in length and as fine as the threads of a spider web. In certain instances, particularly with some of the acid-producing bacteria, the ropy material is tough and doughy. If ropiness is at all pronounced, it is evident when the material is poured or when a spoon is drawn through it. In general, ropiness present in sweet milk or cream disappears as acid develops, whereas that accompanying a definite acid production frequently increases with an increase in acid and then later decreases. Vigorous agitation markedly decreases ropiness. Normal high-fat cream occasionally shows a consistency which suggests a slightly ropy condition to anyone unfamiliar with the product.

Ropy milk should be distinguished from gargety milk coming from certain abnormal udders. With gargety milk the unusual condition is due to the presence of masses of fibrin and leucocytes thrown out by the udder tissues in response to an infection and also to the abnormal composition; with ropy milk the consistency is due entirely to growth of bacteria in what originally was a normal product. Gargety milk shows the abnormal condition when drawn and, if not evident from the appearance of the milk itself, often is recognized by the material

collecting on the filter or in the clarifier bowl, whereas ropiness ordinarily is not present until the milk has stood at least 12 hr.

Importance. Ropiness is one of the common abnormal fermentations of milk and its liquid derivatives. It is encountered most fre-

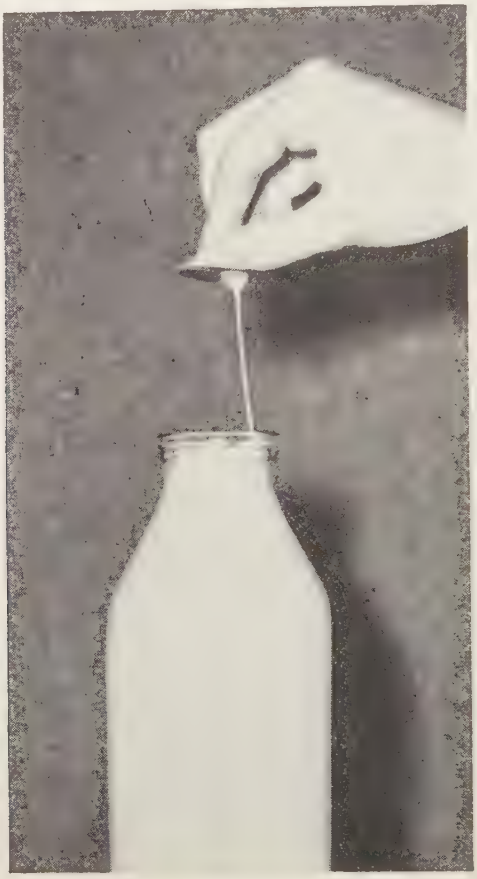


Figure 12. Ropy milk adhering to bottle cap. Causative organism *Alcaligenes viscosus.*

quently and is most serious in market milk where it causes much annoyance to consumers and dealers. Murray [59] described an outbreak of ropiness which affected from 20 to 80% of the bottles. Milk may be delivered in a normal condition and then show ropiness after being held by the consumer. This often occurs when the holding temperature is quite low because some of the organisms producing

ropiness grow unusually well at fairly low temperatures and determine the change taking place there. At higher temperatures they are overgrown by the lactic acid organisms. Often only the cream is ropy and the milk beneath is normal; this is because of the oxygen requirements of certain of the causative organisms, although concentration of bacteria in the cream layer also may be a factor. Milk consumers frequently assume that ropiness is due to disease in the producing animals and, unless the dealer quickly brings the defect under control, loss of patronage may result. The organisms causing ropiness appear to be entirely harmless to persons using ropy milk or cream.

Sweet cream delivered to dairy plants sometimes shows a ropy condition and, as with sweet milk, this is most likely to occur when the holding temperature is rather low. Ropiness occasionally is noted in sour cream and in lactic culture; in these products it usually is due to action of the organisms involved in the acid development.

Causative Organisms. Various organisms have been responsible for ropiness in dairy products. A common one is *Alcaligenes viscosus* which probably was first isolated by Adametz from water. After noting the action of the organism in milk and predicting that it might be the cause of ropiness in this product, Adametz [81] obtained it from ropy milk. It has been isolated repeatedly in Europe and also has been encountered in many sections of the United States. In old cultures there is less acidity than there was in the original milk, but the reaction change occurs slowly. *Alc. viscosus* is one of the ropy milk organisms that definitely requires air; in pure cultures in skim milk there frequently is a membrane at the surface. The organism produces a large capsule that can be demonstrated best in very young cultures since, in older cultures, the capsules from different cells unite and form long threads, leaving the cells free or embedded in the capsular material instead of each having a definite capsule. *Alc. viscosus* grows well at fairly low temperatures, such as 50 F, although growth is faster at somewhat higher temperatures; at 98.6 F growth occurs but ropiness in milk is not pronounced.

Long and Hammer [82] reported that organisms which are like *Alc. viscosus,* except for the failure to produce ropiness in milk and other liquid media, are encountered among organisms isolated from dairy products. The designation *Alc. viscosus* var. *dissimilis* was suggested for them. Some of the cultures which produced no ropiness when first isolated gave ropiness after a number of transfers in milk. Typical *Alc. viscosus* cultures varied widely in extent of the ropiness produced and in the period required to bring about the change; it was noted

that they hydrolyze fat. An organism producing ropiness in milk which is essentially like *Alc. viscosus* except that it eventually develops an acid reaction [83] has been isolated, and also one which differs from typical *Alc. viscosus* in its inability to hydrolyze tributyrin and in its fermentation reactions.[84]

Organisms of the *Escherichia-Aerobacter* group have caused many outbreaks of ropiness. Although most strains belonging to the group do not produce a ropy condition when grown in milk, certain of them do; organisms of the latter class occasionally are isolated from dairy products which show no evidence of ropiness. *Escherichia-Aerobacter* organisms produce a ropy condition in milk by development of capsular material that forms threads through the milk, as with *Alc. viscosus*. Although the organisms produce gas in milk, gas usually is not noted in milk developing ropiness under practical conditions. Acid production of the organisms also appears to be of minor importance, and milk is criticized for the ropiness with no mention of acid. *Escherichia-Aerobacter* organisms have caused ropiness in cream with an acidity of only 0.16%.

Escherichia-Aerobacter organisms causing ropiness in dairy products have been identified on a species basis in comparatively few instances, but the available data indicate various species are involved.

In the *Escherichia-Aerobacter* group, development of ropy cultures from nonropy ones is indicated by the work of Stark and Stark,[85] who found that cultures of *A. aerogenes* could be dissociated into a slimy type which produced ropiness in sterile milk. Dissociation was most quickly induced by addition of an amount of bacteriophage too small to cause complete lysis of the culture. *A. aerogenes* bacteriophage was demonstrated on lettuce, carrots, corn silk, and other plant tissues.

Coccus forms have been responsible for ropy milk. Hammer and Cordes [86] reported an outbreak due to a coccus now designated *Micrococcus cremorisviscosi*. It rapidly digests milk but develops pronounced ropiness before the digestion is evident. O'Droma and Grimes [87] found *Micrococcus pituitoparus* the cause of ropiness in milk from farms and city vendors, particularly during the winter and spring. In milk under favorable growth conditions, the organism seemed to crowd out and almost entirely inhibit development of the bacteria normally present. Ropiness did not appear to be dependent on development of capsules but probably was related to the peptonizing action, with resultant formation of mucins. *M. pituitoparus* commonly was not found in water but may have been carried into surface

waters from plant and soil sources during rainy periods. It was destroyed easily at 145 F for 15 min.

Prouty [88] studied an outbreak of ropiness in commercially pasteurized milk and isolated a thermoduric *Micrococcus* closely related to *M. freudenreichii.* Contamination was traced to one of the farms supplying the milk for pasteurization. Although a detailed study of conditions prevailing on the producing farm was not made, control methods which were put into effect did not overcome the defect. An outstanding characteristic of the organism was its ability to produce ropiness accompanied by an abnormal odor and followed by active digestion of the curd. The organism survived 143 F for 20 to 35 min. M'Kenzie *et al.*[89] studied ropiness in milk that was caused by an unidentified *Micrococcus.* Murray [90] noted that about 15% of the milk producers of each of two dairies supplied milk which became ropy in 24 hr at room temperature; the causative organism resembled *Micrococcus viscosus* but differed somewhat from it.

Organisms Causing Ropiness and Considerable Acid. Ropiness frequently is associated with production of considerable acid. Lactic cultures may develop this condition after varying periods of successful propagation, and ropy sour cream occasionally is encountered. Certain cultures of *S. lactis* (*S. lactis* var. *hollandicus*), *L. casei,* and *L. bulgaricus* show ropiness, and this property appears to be lost, or acquired, with these organisms for no apparent reason although in general a culture maintains either a ropy or nonropy character. The ropiness may be evident before any considerable acidity develops; often it increases for a period and then decreases as the acidity becomes pronounced. The ropiness is due to conditions other than capsule formation. *S. lactis* cultures frequently show some cells with capsules, but the percentage of encapsulated cells is no larger with ropy cultures than with nonropy ones. *L. bulgaricus* apparently does not produce capsules; long chains of cells may tend to make the fermented milk draw out into threads, although rather long chains are present in some cultures that are not ropy. *L. casei* sometimes shows chains of cells but more often does not. It produces capsules in both ropy and nonropy cultures. There is some evidence that the number of organisms per milliliter determines, to a large extent, the presence or absence of ropiness.

Ropiness Due to Associative Action. Buchanan and Hammer [83] reported ropiness in cream that was due to associative action of two organisms, one of which was *S. lactis.* The other organism produced no change in milk in 3 or 4 days, but when grown with certain *S. lactis*

cultures ropiness quickly developed. Ropiness produced by the combination appeared to be due to large numbers of organisms since the plate method and microscopic observation showed that the mixed cultures contained many more organisms than pure cultures of either species. The mixed cultures in milk also gave a slightly lower acidity than pure cultures of *S. lactis*, and this may explain the greater multiplication in the mixtures.

Ropy Organisms of No Practical Importance. Many organisms from a variety of sources have been described as producing ropiness in milk, but most of them have not caused outbreaks and probably never will be of significance since they are overgrown so readily by other species. From the available data it appears that in the United States *Alc. viscosus* and *Escherichia-Aerobacter* organisms are the important species from the standpoint of economic losses due to ropy milk.

Materials Causing Ropiness. The materials responsible for the ropiness developed in milk by certain bacteria apparently are of two types, the true gums or gum-like substances and the mucins. The gums are polysaccharides; the mucins are nitrogenous mucus-like substances. Gums probably are more commonly the cause of ropiness in milk than mucins. Gainor and Wegemer [91] found that the capsular material produced by an organism resembling *Alc. viscosus* was a polysaccharide yielding only fructose on hydrolysis. Jones [92] reported that both *Alc. viscosus* and *M. cremorisviscosi* produced ropiness only when albumin was present. *A. aerogenes* produced ropiness in any medium supporting growth, including a synthetic medium with glycerol as a carbon source; a true polysaccharide was not produced by it,[93] but it did produce an abundance of nucleic acid constituents which were not formed by *Alc. viscosus* or *M. cremorisviscosi*.

Sources of Ropy Milk Organisms. In a ropy milk outbreak the source of the responsible organism is an important consideration. After the organism once is established, infection undoubtedly occurs from utensils and equipment, coats of the animals, etc., but control measures give only temporary relief unless the original source is controlled.

Extensive studies on the flora of aseptically drawn milk show that ropy milk organisms ordinarily are not present in the udder. However, organisms capable of producing ropiness in milk have been obtained from this source, and in an outbreak caused by *M. cremorisviscosi* [86] the organism was found in milk drawn so as to exclude outside contamination, although it was impossible to determine whether the udder invasion preceded or followed the outbreak.

In outbreaks of ropy milk the organisms often come from surface water. One of the common routes of infection is contamination of the coats of the cows from which the organisms then fall into the pail at milking. Harding and Prucha [94] reported that in two instances in connection with an outbreak studied by them material collected from the flanks and udders of cows produced characteristic ropiness when added to sterile milk. In some cases outbreaks of ropy milk have promptly followed flooding of the land on which the producing animals were pastured, and outbreaks have been stopped simply by keeping the animals from surface water. In addition to water standing in pastures, water from troughs, milk cooling tanks, and directly from wells must be considered.

When ropy milk organisms get into utensils they may live over from one milking to the next with the cleaning methods practiced on many farms. Samples collected on farms delivering milk containing ropy milk organisms have indicated the organisms were in the utensils; [94] at one farm the organisms were isolated repeatedly from utensils and from water in the cooling tank. Ward [95] found *Alc. viscosus* the cause of ropiness in two creameries in both of which the organism appeared to come from insufficiently scalded utensils. Greater care in scalding brought the trouble to an end. In one outbreak three different lots of sterile milk picked up the organism from the surface of a strainer.

Stark and Foter [96] obtained results indicating cow feeds are important sources of ropy milk organisms, since alfalfa hay, soy bean meal, and cottonseed meal contained many thousands per gram; certain other feeds did not yield the organisms.

Controlling Ropiness. In combating an outbreak of ropy milk, sterilization of all utensils and equipment coming in contact with the milk, including bottles and cans, is one of the first steps. The cooling tank may be a source of organisms through water splashing into the milk. Stables should be given a thorough cleaning and disinfection since in some instances the milk apparently is contaminated from the air of stables. Flanks and udders of the cows can be wiped with a cloth dampened with disinfecting solution and continued contamination of the coats of the animals prevented by keeping them from surface water. Control of an outbreak often is difficult, and there may be a recurrence when the precautions taken are neglected, even for a short period. In certain cases ropy milk has been eliminated only after a long period of attention to many details.

Heat Resistance of Organisms. Although certain ropy milk organisms have been reported to survive pasteurization, the process is an effective means of control, provided the heated milk is not recontaminated. Outbreaks of ropiness in raw milk supplies have been controlled immediately by introduction of pasteurization.

Harding and Prucha [97] investigated the heat resistance of cultures isolated from outbreaks of ropy milk, including both *Escherichia-Aerobacter* organisms and *Alc. viscosus.* When heated in bouillon, some of them survived 10 min at 140 F but not at 149 F. In ropy milk a number of cells survived 140 F for 20 min but not for 30 min.

Hammer and Hussong [98] studied the heat resistance of *Alc. viscosus* from three sources: (1) An outbreak of ropiness in pasteurized milk, (2) butter which showed ropy moisture droplets at the surface and which was made from pasteurized cream, and (3) ropy whey obtained from pasteurized milk set for cheese. Cultures of various ages failed to survive 142 F for 3 min. Three strains of *A. aerogenes* that produced ropiness in milk varied in their heat resistance. With one strain organisms from an old milk or agar culture survived 145 F for 10 min, but those from young milk cultures did not; with a second strain organisms from young or old milk or agar cultures failed to survive 142 F for 3 min; with a third strain organisms from old milk or agar cultures regularly resisted 145 F for 10 min and, in some instances, for 20 min although organisms from young cultures failed to resist these exposures.

Ropiness in Pasteurized Milk. Extensive outbreaks of ropiness occur in pasteurized milk. In general, they are due to contamination from plant equipment after the heating, especially from equipment that is difficult to sterilize properly, although some other irregularity in the plant operation also may be responsible. Ropy milk organisms have been found in detergent and rinse solutions of bottle washers.[99] Also, they appear to be difficult to wash from bottles. Outbreaks in pasteurized milk can be stopped by thorough cleaning and disinfection of the plant and equipment, but recurrence frequently is noted. The presence of ropy milk bacteria in the raw milk coming to the plant is a possible source of the organisms.

In investigating an outbreak that had continued for more than 2 yr, Kelly [100] found the ropiness was due to organisms gaining entrance to the milk through pipes that were used first for raw and then for pasteurized milk. The difficulty was aggravated by bringing into the plant milk that was returned from stores and wagons; one lot of re-

turned milk contained 32,000 ropy milk bacteria per ml. When the returned milk was kept out of the plant and other faults corrected, the organisms were eliminated from the equipment by use of steam, hot water, and chlorine.

Isolation of the Causative Organism. Isolation of the causative organism from ropy milk or cream usually can be accomplished by plating when the defect is due to such organisms as *Alc. viscosus* or an *Escherichia-Aerobacter* species. With these types, the viscous character of the colonies makes them easily detected in a mixed flora. Some of the plates poured should be incubated at 70 F (21.1 C) since with *Alc. viscosus* and *Escherichia-Aerobacter* species the colonies often are less ropy at 98.6 F (37 C) than at lower temperatures. If the ropiness may be due to *S. lactis* var. *hollandicus* or a *Lactobacillus*, it is advisable to plate on a suitable agar in addition to the usual medium and to pick colonies into sterile milk and incubate at 70 F to detect the ropiness.

When samples of normal milk are to be examined for ropy milk organisms, it is advisable to hold them at relatively low temperatures, such as 50 F, and observe for ropiness after various holding periods. At these temperatures certain ropy milk organisms grow more rapidly than most other species present and thus are detected easily.

Distribution of Ropy Milk Organisms. The common presence of ropy milk organisms in milk, even when ropiness does not develop, was shown by Ward [101] who found that the milk furnished by 42 patrons out of a total of 307 delivering milk to 3 plants became ropy when held 4 days at 40 to 45 F; no complaints of ropy milk were received at the plants. It was noted that in several plants ropy milk organisms frequently were present on any utensil that had been washed but not scalded and in various pieces of equipment. Harding and Prucha [97] reported that holding individual samples from 140 farms supplying a bottling plant having trouble with ropy milk yielded ropiness one or more times with the milk coming from 116 of them. Kelly [100] examined milk from farms and found 17 of 150 samples contained organisms producing ropiness. A more detailed study of milk from 31 farms showed that milk from 6 became ropy in 2 days at 58 F. Jones [102] isolated 440 ropy cultures by plating over 3,500 samples of raw milk and picking colonies. The cultures included three stable, highly ropy species, *Alc. viscosus, A. aerogenes,* and *M. cremorisviscosi.* Ropy *S. lactis* cultures were isolated, but they eventually lost their ropy character.

Organisms which produce ropiness in milk often are isolated from milk or cream which either is normal or shows abnormal conditions other than ropiness. It is evident that ropy milk organisms can be present in these products without causing a change in consistency.

Use of Ropy Milk. Ropy milk resulting from the growth of *L. bulgaricus* has been added to buttermilk that "wheys off" rapidly to delay settling of the casein. Some of the fermented milk consumed in certain areas, particularly the Scandinavian countries, is ropy and, while it is not prized because of the ropiness, the condition is not objectionable. Macy [103] isolated a *Streptococcus* producing ropiness from a ropy milk beverage used by certain Finns in the United States.

SWEET-CURDLING FERMENTATION

In the sweet-curdling fermentation, organisms curdle milk or cream through production of a coagulating enzyme comparable to rennin. Milk or cream that curdles at ordinary temperatures with an acidity too low to account for the coagulation ordinarily has been changed by such an enzyme. Some sweet-curdling organisms produce little or no acid, but others form considerable amounts after the curdling has taken place so that in detecting sweet curdling the acidity should be determined as soon as coagulation occurs.

Importance of Sweet Curdling. Sweet curdling is one of the more uncommon milk fermentations although it is by no means rare. Milk and cream delivered to dairy plants may show this change, and it sometimes is observed by the retail purchaser of the products, especially when they are held for considerable periods after pasteurization. Sweet curdling is encountered most frequently during the warm months or when the product involved is held at fairly high temperatures.

Cream with a normal acidity occasionally coagulates during pasteurization in butter plants, and this has been assumed to be due to the presence of small amounts of coagulating enzyme whose activity is increased at comparatively high temperatures. The work of Frazier [104] confirms this idea. He found that although small amounts of milk culture of a sweet-curdling organism inhibit heat coagulation over a small range, large amounts hasten it. When purified bacterial rennin was used, there was no influence on the heat coagulation if the milk was heated immediately upon addition of the enzyme, but the heat coagulability of the milk was increased if the rennin was

allowed to act for a time. The optimum temperature for most rennins was found to be 104 to 109.4 F, whereas 140 F for 15 min destroyed them.

Although the sweet-curdling fermentation is not common under practical conditions, almost any lot of milk contains organisms capable of causing this change. Their failure to develop ordinarily is due to overgrowth by the lactic acid bacteria, and it requires favorable conditions, with perhaps few competing organisms, for sweet curdling to occur.

Causative Organisms. The organisms capable of sweet curdling milk are extremely varied in their characteristics and belong to widely separated genera; the general types are:

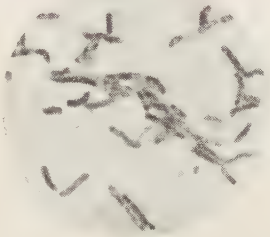


Figure 13. Spore-forming sweet curdler.

1. Aerobic Spore-producing Bacteria. The aerobic spore-producing bacteria include various species that are pronounced sweet curdlers. Organisms of this type regularly are present in milk and cream and can be isolated by heating to destroy the nonspore formers, holding under aerobic conditions at a temperature favorable for growth, such as 77 to 98.6 F, until curdling occurs, and then plating. In general, these organisms enter milk from dust, dry manure, etc., the spores enabling them to resist the desiccation to which such materials are subjected. The aerobic spore producers occasionally cause sweet curdling under practical conditions, especially in pasteurized milk or cream held at comparatively high temperatures.

Lawrence and Ford [105] studied the aerobic spore formers in the Baltimore milk supply and reported that they believed *Bacillus albolactis* to be the common cause of the clotting and peptonization occasionally seen with boiled milk, and also that it undoubtedly is a contributing factor in the changes seen in milk heated to temperatures such as 140 to 149 F. This organism was encountered less frequently in samples of milk heated with various exposures than either *Bacillus cereus* or *B. subtilis*. The aerobic spore formers involved in the sweet curdling of milk and cream present difficulties from the standpoint of classification. Ordinarily, classification on a species basis is not necessary in dealing with the organisms in these products.

2. Nonspore-producing Rods. A number of nonspore-producing rods are capable of sweet curdling milk. With most of them the curd formed is very soft and inconspicuous, and the striking thing is pro-

Univ. of Arizona Library

teolysis. Organisms belonging to the genus *Proteus* are representative of this general group and illustrate the usual type of change. The nonspore-producing rods that cause sweet curdling often are encountered in studying the bacterial flora of milk and cream and, apparently, come from a variety of sources; they are rarely, if ever, responsible for sweet curdling under practical conditions.

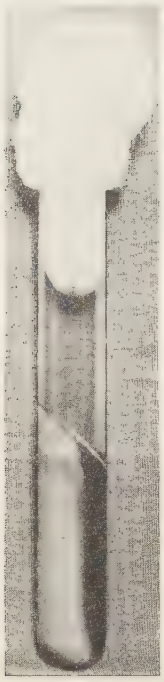


Figure 14. *Streptococcus liquefaciens* in milk, showing proteolysis following coagulation.

3. Coccus Forms. Various species of cocci, with widely differing characters, sweet curdle milk. One of the more important is the lactic acid organism *S. liquefaciens* that sweet curdles milk in which it is growing before the acid production is conspicuous. This organism has been found responsible for sweet curdling in a number of instances. Some of the micrococci sweet curdle milk but bring about the change rather slowly and are unimportant as a cause of the fermentation under practical conditions.

4. Yeasts and Molds. Various yeasts and molds can sweet curdle milk but do it so slowly that they are of no practical significance.

PROTEOLYSIS

Proteolysis is the process by which casein, or some insoluble casein derivative, is broken down to water-soluble compounds through the action of organisms. Extent of the decomposition varies greatly with different species; sometimes it is almost complete and very evident, and in other instances it is slight and can be established definitely only by chemical determination of the amount of soluble nitrogen present. The decomposition products formed usually are a mixture of various groups of compounds and include peptones and related bodies, amino acids, ammonia, and even free nitrogen.

Relationship of Proteolysis and Sweet Curdling. The organisms causing conspicuous proteolysis are sweet curdling forms, and pronounced proteolysis rather regularly follows sweet curdling. It appears that, with certain organisms at least, the incubation temperature determines whether proteolysis or sweet curdling is the more evident. Sarkaria and Hammer [106] noted that, with the organisms studied by them, coagulation was more conspicuous at 98.6 F than at room tem-

perature. Observations of various investigators suggest that some of the organisms which sweet curdle milk when first isolated lose this property on cultivation and then have increased proteolytic powers. However, the effect of various salts on the activity of bacterial rennin may be a factor tending to cause variations in the results obtained with an organism.

The frequency with which proteolysis and sweet curdling of milk are associated in the characters of organisms has led to the suggestion that they always occur together and that, occasionally, one of the changes is overlooked in studying an organism because it is not pronounced at the temperature used. Cultures that have been described as proteolyzing milk without any mention of sweet curdling sometimes show definite sweet curdling when grown at suitable temperatures.

The proteolyzing action of certain organisms is evident on plates poured with media containing milk or casein. In some cases organisms that actively proteolyze on plates have only limited action when inoculated into milk. This may be due to the abundance of sugar in the milk which the organisms use in preference to the casein.

Bitterness Following Proteolysis. Proteolysis of milk and cream often is accompanied by development of a bitter flavor. This is due to the presence among the protein decomposition products of such bodies as peptones that are bitter. Sometimes bitterness is evident in milk or cream before either proteolysis or sweet curdling is conspicuous. Under practical conditions this occurs most frequently when the products are held at relatively low temperatures, such as 50 F, and *S. liquefaciens* often is the causative organism.

LIPOLYSIS

Various organisms hydrolyze fat through the production of lipase with the liberation of fatty acids and glycerol, and the change is referred to as lipolysis. The lower fatty acids in milk fat, especially butyric and caproic, have conspicuous odors that are objectionable in most dairy products. When the odors of the lower fatty acids are evident in milk, cream, butter, etc., the condition commonly is referred to as rancidity. Lipolysis due to organisms should be distinguished from that caused by the lipase normally present in milk.

Using the general procedure employed in detecting lipolytic organisms (see p. 16), cultures can be tested for their lipolytic powers by inoculating them on plates containing dispersed fat; observations

are made directly or after flooding the plates with an indicator, such as nile blue sulfate. An organism also can be tested for lipolytic action in other ways. It can be inoculated into sterile cream and the cream examined from time to time for the odor of the lower fatty acids. Also, it can be inoculated into sterile or pasteurized cream, the cream churned, and the butter examined at frequent intervals; the butter should be left unsalted since many organisms are inhibited by salt, and it should not be incubated above 70 F in order to avoid any serious influence on the physical condition of the butter.

Some lipolytic organisms apparently assimilate certain of the products of fat hydrolysis and, in this way, accumulation of various compounds may be delayed. Jezeski *et al.*[107] found evidence, with four bacterial cultures, that oxygen consumption in the presence of fats may be due to utilization of glycerol resulting from the lipolysis. The lower fatty acids are rather toxic for many species, especially in such a product as butter, and sometimes cause a rather rapid decrease in numbers of organisms.

Various organisms can hydrolyze fat and certain molds are especially active. Collins and Hammer [108] isolated 159 lipolytic bacterial cultures from different sources, such as water, air, dairy plant equipment, and dairy products and found a variety of species. Common lipolytic bacteria are *Pseudomonas fragi,*[109] *Achromobacter lipolyticum,*[110] and *Pseudomonas fluorescens.* Many of the actively lipolytic organisms also are proteolytic. Anderson [111] studied an organism characterized by its powerful fat-splitting action, weak action on protein, inability to act on sugars, and rapid growth at refrigeration temperatures. It liberated caproic, isocaproic, and caprylic acids from refrigerated cream and caused a peculiar irritation when the cream was swallowed. Rate of growth of the organism in milk at 39.2 F was such that 1,500 bacteria per ml after 11 hr could become 1,000,000 after 60 hr and 130,000,000 after 110 hr. The organism now is designated *Achromobacter lipidis.*

Richards and El-Sadek [112] determined the fatty acids liberated during the development of rancidity in 12 samples of butter, 6 naturally rancid and 6 made rancid by cultures of molds and bacteria. The mixtures of acids were separated into volatile, solid, and liquid. Glycerides of the volatile acids apparently were attacked readily, especially by molds. The rancidity was roughly proportional to the amounts of these acids. It appeared that the solid acids were liberated least readily by both molds and bacteria; they probably were mainly lauric and myristic with little palmitic or stearic. The liquid

acids formed 77.2 to 86.9% of the total free acids and were liberated readily from their glycerides; oleic and linoleic probably were most abundant.

Milk and cream lipolysis due to bacteria is noted most commonly when the products are held at relatively low temperatures, such as 45 F. Under these conditions the lactic acid organisms are greatly inhibited and certain lipolytic species, especially *Ps. fragi*, develop relatively rapidly because of their psychrophilic character. Lipolytic bacteria are isolated most easily by holding raw milk or cream at a rather low temperature until rancidity develops and then plating on a differential medium.

FLAVOR FERMENTATIONS

Many organisms that grow in milk definitely affect the flavor; the influence is much greater with some species than with others. Flavor includes the impressions obtained through the senses of taste and smell. These senses are so interrelated that impressions obtained through the sense of taste ordinarily are not differentiated from those obtained through the sense of smell, and what often is taken to be the taste of a product is in reality a combination of taste and odor.

Importance of Flavor Fermentations. Changes in the flavor of raw milk and cream through bacterial action are very common and are related to poor production methods or inadequate cooling. Milk coming to plants is examined for off odors, many of which are bacterial, and butter factories often have high standards for cream.

Abnormal flavors in milk and cream may be due to causes other than the action of organisms, such as feeds, condition of the producing animals, enzymatic action, etc. (see p. 27), and these should be distinguished from those due to organisms.

Flavors Produced by Organisms. Flavors produced in milk through growth of organisms are extremely varied. An acid flavor is the most common, but many others are frequent. Flavors, such as feed or barny, that suggest other causes may be due to organisms. For example, in one instance milk delivered by a certain producer frequently showed a flavor that was thought to be due to feed, but investigation proved it was the result of growth of an organism; the flavor was common in the night milk that was held for a period on the farm but was absent in the morning milk which was delivered soon after milking. Barny and other objectionable flavors apparently may be due to growth in milk and cream of the organisms which are re-

sponsible for decompositions in manure. Such specific flavors as soapy and turnipy occasionally are due to activity of organisms, instead of the expected causes.

A malty flavor often is present in milk and cream as a result of growth of *S. lactis* var. *maltigenes* and may be noted regularly in products from certain farms. *Escherichia-Aerobacter* organisms frequently cause unclean flavors. Bitterness is due to a variety of organisms. Organisms producing fishiness in milk and cream have been isolated. Fellers [113] reported that *Actinomyces* organisms may cause an objectionable bitter-moldy taste in milk after some hours. Two outbreaks of bitter-moldy milk in one dairy were traced to the presence of large numbers of the organisms; the source probably was hay and straw.

Occasionally, milk and cream have an odor suggestive of potatoes. From such cream Brannon [114] isolated an organism which reproduced the odor in freshly separated cream. Olson and Hammer [115] studied an outbreak of potato odor in milk from a certain farm and found the causative organism was *Pseudomonas graveolens* which originally was isolated from musty eggs. The organism failed to grow at 98.6 F. In aseptic milk inoculated with it and held at about 46 F, the count increased from 12,900 per ml immediately after inoculation to 5,500,000 after 96 hr when the potato odor was very evident. *Pseudomonas mucidolens* [115] is another organism which has been isolated from cream that had developed a potato odor at a relatively low temperature; it also originally was isolated from musty eggs.

Claydon [116] reported an outbreak of medicinal flavor in pasteurized market milk that was caused by *A. aerogenes.* It occurred during late spring and considerable defective milk was returned to the plant. The organism isolated reproduced the typical defect readily in pasteurized and sterilized milk. Hand-washed bottles appeared to be the immediate source of the organism and improved sanitation in the bottle cleaning controlled the outbreak.

Many of the off flavors produced in milk and cream by micro-organisms are rather temporary and are replaced by others that often are still more objectionable. With an extended holding period, there are certain sequences that occur with considerable regularity; for example, a fishy flavor in cream commonly is followed by a metallic flavor. In sour cream that has been held for extended periods cheesy, rancid, and yeasty flavors are common.

The flavors produced in milk and cream by micro-organisms occasionally are very pleasant, although decidedly abnormal; fruity flavors

are perhaps the best examples. In general, these pleasant flavors are quickly replaced by others that are objectionable.

The temperature of holding has a great influence on the flavors that develop in milk and cream. Temperatures such as 32 F prevent growth of *S. lactis* and thus permit activity of certain organisms sensitive to acid that are capable of causing protein decomposition and fat hydrolysis. Small differences in the holding temperature often result in wide differences in the flavor produced because of variations in the comparative growth rates of the organisms present.

In some instances organisms which are classified as belonging to the same species produce different flavors in milk. This indicates that a small variation in the action of an organism, which appears unimportant in its general reactions and classification, may be significant from the standpoint of flavor production.

Associative Action. With the flavor fermentations, as with so many other changes in milk and cream, associative action of organisms is important. Flavors that, from their manner of development, evidently are the result of bacterial action sometimes cannot be reproduced with pure cultures, whereas mixed cultures cause the condition. The difficulties encountered in attempting to develop certain flavors experimentally suggest that the balance between the causative organisms often is a delicate one.

COLOR FERMENTATIONS

Action of organisms may bring about changes in the color of milk and cream. Although such fermentations have been noted occasionally in Europe, they are of little practical importance in the United States.

Many organisms commonly present in milk produce colored colonies, especially yellow, orange, or red, on various solid media, but development of color under these conditions is quite different from color production in a typical color fermentation. Some of the species forming colored colonies show no color when grown in milk; others show only a colored sediment or surface ring consisting of a mass of cells. In a typical color fermentation, the color is distributed through considerable portions of the milk and is not confined to masses of growth. In general, the color changes involve the surface of milk or cream because of the oxygen requirements of the causative species. With the blue fermentation due to *Pseudomonas syncyanea* (*Pseudomonas cyanogenes*) and possibly with other color fermentations, associative action is important.

Blue Fermentation. Although various organisms have been responsible for blue coloration in milk, the one most frequently isolated appears to be *Ps. syncyanea.* The color produced, which often is intense, is formed only in the presence of acid.

A small outbreak [117] of blue milk was reported by an Iowa dealer supplying 18 families. The color was observed by the consumers 3 times, twice in one household and once in another. The 2 families were related and kitchen utensils frequently were exchanged. The color was not observed on the producing farm, except in one instance when the container used was brought from one of the homes in which the blue color had been noted. Conditions on the farm were normal and the organisms in the milk apparently came from the usual sources. In all instances, the blue color was observed in sour milk and appeared in areas at the surface. When milk showing the blue color was examined, the organism found most abundant was *S. lactis,* but another type, which proved to be *Ps. syncyanea,* also was present in considerable numbers. Neither species alone gave a blue color, but a mixture of the two did. The history of the outbreak suggests that the original infection of the milk with *Ps. syncyanea* occurred in one of the households noting the defect. After the organism was established, it probably was carried to the other house through the transfer of utensils.

Characteristics of *Ps. syncyanea.* The striking characteristic of the blue fermentation of milk due to *Ps. syncyanea* is that it does not occur except when acid is present. In pure culture in milk the organism fails to yield a blue color, but a brownish tinge may develop. *Ps. syncyanea* tends to produce an alkaline reaction in milk, and in combination with an acid-producing organism it may grow longer than it would alone since the acid neutralizes the alkaline products formed. The organism yields material that presumably acts as an indicator and shows a definite blue color in the presence of acid; however, addition of acid to milk in which *Ps. syncyanea* has grown alone does not result in the color change. Importance of acid in the color production is shown by development of both acid and color in pure cultures of the organism in dextrose bouillon.

Blue Milk Due to Nonbacterial Causes. Blue color in milk may result from causes other than growth of bacteria. A low content of solids and the eating of certain plants by the cows are among such causes; with these, the bluish tinge is evident when the milk is drawn although it is more pronounced after the fat separates.

Yellow Fermentation. Yellow color sometimes is produced in milk through bacterial action, and the most common causative organism

appears to be *Pseudomonas synxantha.* This species has been found in the United States, but only incidentally; apparently, it has not been noted under practical conditions but has been isolated from milk that has stood at room temperature until yellow color developed at the surface of the cream.

With *Ps. synxantha* [118] the yellow color is pronounced in the cream layer of milk, and the typical color change appears to be associated with the fat. The organism proteolyzes milk and the digested material may have a yellow tinge, but a conspicuous color is found only in the fat layer. Sometimes black granules also are present in the fat; these consist of some decomposition product formed by the organism. *Ps. synxantha* produces a disagreeable flavor in milk, and the digested material may become viscous. The yellow color of butter can be intensified by growing *Ps. synxantha* in the cream from which it is made, but the color development is accompanied by a disagreeable flavor.

Various organisms digest milk with formation of yellow color, but these cannot be regarded as of significance since the digestion usually requires considerable time and involves a pronounced change in appearance and composition of the milk.

Yellow Milk Due to Nonbacterial Causes. The normal milk of certain animals, especially Guernseys, shows a yellow color which varies in intensity with the season. Such feeds as carrots and mangels sometimes cause a pronounced yellow color in milk. When there is an udder infection the milk may have a yellow tinge.

Red Fermentation. Red color is produced in milk by a number of bacterial species, such as *Serratia marcescens,* growing in pure culture; although the organisms occasionally are present in milk and cream, more rapid growth of other forms usually prevents their development.

Sour milk or cream, when held for considerable periods, may show red spots on the surface which are colonies of pink yeasts; the most common of these is *Rhodotorula glutinis.* Pink yeasts frequently are present in milk and cream and are not restrained by the usual acidities developed; however, they produce red spots only occasionally because of their comparatively slow growth.

Red color in milk due to action of organisms must be distinguished from red color due to presence of blood. A ruptured vessel in the udder may result in blood in the milk and, if it is present in considerable amounts, the color of the milk is changed. On standing, some of the erythrocytes settle to the bottom while many are carried up with the cream, especially that making up the lower portions of the cream

layer. Blood sometimes is present in milk in such small amounts that the color is not perceptibly changed, but such a condition often can be detected by centrifuging a small volume of the milk, when the erythrocytes are thrown into a layer at the bottom of the centrifuge tube.

A red tinge in the glass occasionally has been the cause of a bottle of milk appearing to have a pink color; the cream especially appears pink since it provides a better background for the slight color in the glass.

YEASTS AND MOLDS IN MILK

Although bacteria are primarily responsible for the fermentations of milk and cream under practical conditions, yeasts and molds normally are present in the products. The numbers per milliliter vary widely, depending on conditions of production and holding. With long holding at comparatively high temperatures, the organisms are relatively numerous, owing to their ability to grow in the presence of considerable acid, and in the extreme condition masses of growth are evident on the surface of acid milk or cream.

Yeasts. The yeasts in milk and cream are of various types. Although certain species, such as the lactose fermenters and some proteolytic types, produce changes rapidly, many of the yeasts are characterized by their slow action on milk. On the surface of sour cream, cottage cheese, etc., yeasts frequently produce colonies, and with the pink yeasts these are conspicuous.

Certain yeasts can be used to produce commercial products from whey and other waste milk derivatives. Under proper conditions *C. pseudotropicalis* gives satisfactory yields of ethyl alcohol from the lactose fermented.[119] Rogosa *et al.*[120] obtained yields averaging 90.73% in the laboratory and as low as 84% in the plant. The optimum temperature for the fermentation was 91.4 to 93.2 F and the *p*H 4.7 to 5.0. Wilharm and Sack [121] found that different strains of lactose-fermenting yeasts incubated at 86 F in whey containing 10, 20, or 30% added lactose produced 6.5 to 7.5% alcohol. Up to 90%, 75 to 80%, 60 to 76%, and 37 to 40% of the lactose was fermented in ordinary whey and in whey containing 10, 20, and 30% added lactose, respectively. Shaking whey during incubation reduced the time required for the fermentation.

Various yeasts have been used to produce feed yeast from dairy byproducts.[122] Graham *et al.*[123] noted that *C. pseudotropicalis* was the most active of the organisms tested in utilizing cheese-whey solids as

food in aerated culture. Supplementary nutrients, such as urea or ammonium salts, stimulated growth.[121] In pilot plant tests the organism completely utilized the lactose in whey in 24 to 48 hr. A whey-yeast mixture dried on an atmospheric roll yielded a nonhydroscopic powder containing 35.6 to 40.2% protein and 17 to 20% fat. With most strains of lactose-fermenting yeasts, Müller [125] found the optimum conditions for growth were 89.6 to 95 F and a *p*H of 4.5 to 5. Using aeration, the maximum production of dry yeast from rennet whey (5% lactose) was 2.4 to 2.7%.

In processes involving the growth of yeasts in waste dairy products, the cost, compared to the selling price of the product, is the important consideration. Use of yeasts for the disposal of waste dairy products also has been suggested. Porges *et al.*[122] reported such a method is not feasible because the oxygen-demanding substances which remain after the separation of solids still have much of the original pollution load.

Molds. The molds in milk and cream also are of various types; often they represent contamination from the air. Active growth involves relatively few species and occurs primarily at the surface because of oxygen requirements. The species that most often grows on milk and cream is *Geotrichum candidum* (formerly *Oidium lactis* or *Oospora lactis*), and it is regarded as the common mold of milk. With extended growth, it forms a heavy wrinkled membrane that is very characteristic.

Thom [126] studied the general manner of growth of *G. candidum* on dairy products and the method of reproduction. Recent studies indicate great variations in cultures of the organism. Macy and Gibson [127] studied 61 cultures isolated from butter and found differences in colony size and appearance, size of conidia, growth temperatures, and heat resistance. All of the cultures liquefied gelatin and hydrolyzed cottonseed oil and butter fat, but none of them formed acid in litmus milk. The flavors produced in skim milk, whole milk, cream, and lactic culture were not pronounced, except with 6 cultures which gave fruity or cheesy flavors.

Purko *et al.*[128] also noted variations in cultures classified as *G. candidum*. Eight of nine cultures caused rapid and extensive hydrolysis of the fat in cream, and one hydrolyzed it rather slowly. Liberation of the water insoluble fatty acids decreased in direct proportion to the added lactic acid content of the cream. Nelson [129] found filtrates from cultures of *G. candidum* contained a true lipase which was active at *p*H 5 to 8 over a range from 68 to 98.7 F; maximum activ-

ity occurred at 86 F in a substrate buffered at *p*H 6.0. Naturally occurring mixed triglycerides were more readily hydrolyzed than synthetic triglycerides or other esters.

In the trials of Friedemann,[130] *G. candidum* in a medium prepared from 0.3% meat extract, or an infusion of beef muscle, to which was added 1% peptone, 1 to 1.8% disodium phosphate crystals, and 0.9% dextrose yielded about 70% lactic acid on the basis of the sugar consumed. Approximately one-fifth of the C_3 intermediates from the

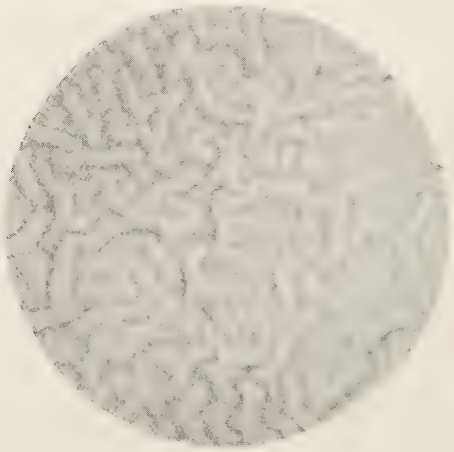


Figure 15. Wrinkled surface growth of *Geotrichum candidum* on cream. Courtesy R. V. Hussong.

sugar were further metabolized to formic acid, acetic acid, and ethyl alcohol.

When growing on the surface of sour milk or cream, *G. candidum* tends to lower the acidity by using some of the lactic acid in its metabolism and by neutralizing some of it with certain growth products. The reduction in acidity eventually may permit development of other organisms, especially bacteria, that were restrained by the higher acidity.

Because of the frequency with which *G. candidum* is present in milk, its source is of importance. Garrison[131] reported that cow manure is its major source on dairy farms. It is distributed widely by manure to various places about the barn and surroundings. Dust raised in the barn by grooming the cows usually contained the organism in significant numbers. Dust from hay and bedding contained relatively small numbers. On farms producing cream, the organism

was isolated from 80.6% of 36 separators, 76.7% of 60 milk pails, and 65.2% of 23 strainers. On farms marketing milk, the milk tubes and teat cups of 5.1% of 39 milking machines yielded the organism. It was detected in various feeds, soil, and water.

G. candidum is destroyed easily by heat. Tanner and Hofer [132] found with 224 cultures from a wide area that none survived 145 F for 30 min. Of 144 cultures exposed in cream to 135 F for 30 min, 8 survived; 125 F for 30 min did not kill any of the cultures.

Figure 16. *Geotrichum candidum,* showing germinating spore and segment of mycelium.

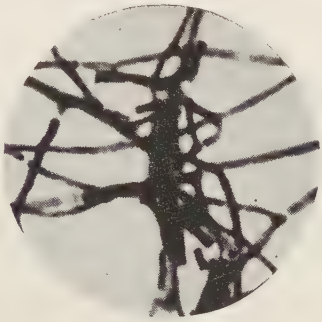


Figure 17. *Geotrichum candidum,* showing developed mycelium.

Popova and Puchkova [133] used *G. candidum* for the biosynthesis of fat from whey. Since the organism has antibiotic properties, it does not require sterile conditions. A good yield of mycelium required ample air supply, active evaporation, and proper nutrients (ammonium dihydrogen phosphate, sodium chloride, asparagin, and urea). Lipase, the chief enzyme involved in the fermentation, governs both fat synthesis and lipolysis. To avoid excessive losses by lipolysis, an oxidizing agent was added. The proteolytic enzymes of the organism also have an important function in the biosynthesis of fat.

NUMBERS OF ORGANISMS REQUIRED TO PRODUCE DEFINITE CHANGES IN MILK

Milk may contain large numbers of bacteria per milliliter without showing abnormalities, and this suggests that, in general, large numbers of organisms are required to produce definite defects. Hammer and Hix [134] inoculated aseptic or sterile milk with various organisms

and made plate counts as defects developed. When an increased acidity recognizable by the sense of taste was produced by S. *lactis* in aseptic milk, the counts varied from 30 to 90 million per ml (7 trials); when the milk was regarded as sour, the counts ranged from 53 to 166.5 million (4 trials). A slightly ropy condition produced by *Alc. viscosus* in sterile milk in thin layers required from 15.5 to 44 million organisms per ml (19 trials); with a very ropy condition the counts ranged from 450 to 786 million (4 trials). Comparable conditions produced by *A. aerogenes* involved counts of 11 to 49 million (7 trials), and of 290 to 475 million (37 trials), respectively. Bitter flavor in aseptic milk was produced by an organism characterized by the development of this defect with counts from 10 to 66 million per ml (10 trials). When a sweet curdler produced slight coagulation in sterile milk, the counts ranged from 1.25 to 4.9 million per ml (21 trials), and when there was firm coagulation the counts varied from 15.9 to 56 million (26 trials). A faint odor was produced in sterile milk by an unidentified organism with counts of 14 to 57 million per ml (20 trials). In the tests the sweet-curdling organism produced a change in milk with the smallest number of organisms.

Other data also indicate that relatively large numbers of organisms ordinarily are required to produce definite changes in milk. Curran [135] found with a sweet curdler that coagulation of milk occurred when a population of 10 to 40 million per ml was attained. Olson and Hammer [115] noted that aseptic milk inoculated with *Ps. graveolens* had a pronounced potato odor with a count of 5.5 million per ml. Gould and Jensen [136] reported that microscopic clump counts on milk samples showing slight acid-associated flavors ranged from 1.5 to 10 million.

Plate counts of the yeasts in cream showing various conditions have been noted as follows: Cream with a yeasty odor 1.6 to 2.2 million per ml; cream with a decidedly yeasty odor 10.6 to 12.8 million; and foaming cream 6.3 to 11.2 million.

In considering the number of organisms required to produce a change in milk, the uneven distribution of organisms under practical conditions must be recognized. When ropiness is present only in the cream layer of a bottle of milk, the number of bacteria per milliliter in the cream is high, whereas in the milk beneath there may be relatively few organisms. Because of the comparatively small volume of the cream, the count on the milk after thorough distribution of the cream may be relatively low.

CHARACTERISTICS OF MILK ABNORMALITIES DUE TO ORGANISMS

Abnormal conditions in milk due to growth of organisms have certain characteristics that ordinarily render them distinguishable from abnormalities due to other causes and, frequently, consideration of the general nature of an unusual condition makes it possible to determine whether organisms are responsible. The more important of the characteristics are as follows:

1. The abnormal condition is not present when the milk is drawn. A defect in milk, such as ropiness or an abnormal color, that is due to activity of organisms requires an opportunity for growth; comparable abnormalities due to condition of the animal are present when the milk is drawn. A flavor caused by action of organisms is not present at the time of milking; one due to feed of the animal is present. When this character is used in connection with flavors in milk, the possibility of absorption from the surroundings must be recognized.

2. The abnormal condition usually increases on standing. In general, as a result of increased growth of the causative organism, the abnormal condition becomes more conspicuous. For example, ropiness becomes more pronounced with longer holding if the temperature remains favorable for the organisms. Eventually, there may be a disappearance of any abnormal condition, whether due to organisms or other causes, because of extensive growth of various species.

3. In many instances the abnormal condition can be inoculated from one lot of milk to another. Organisms causing an abnormality in milk often produce the same condition when a bit of the milk is transferred to a fresh lot, preferably sterile milk. However, a number of factors, such as growth of organisms other than the causative species and use of unsatisfactory temperatures, can be responsible for failures in such trials. When reproduction of an abnormal condition is attempted, it is advisable to work with cultures, either pure or in known mixtures, rather than with the abnormal material direct.

Important Exceptions. Rancid and bitter flavors in the milk of certain animals advanced in lactation and oxidized and tallowy flavors caused by various factors show certain characters suggesting that they are due to organisms; for example, they increase on standing and commonly are not present when the milk is first drawn. However, these flavors are so characteristic that they are recognized readily.

REFERENCES

1. *Can. J. Technol.*, **30**, 130 (1952).
2. *Compt. rend.*, **5**, 822 (1837).
3. *Compt. rend.*, **45**, 915 (1857).
4. *Quart. J. Microscop. Sci.*, 177 (1878).
5. *J. Dairy Sci.*, **4**, 1 (1921).
6. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 65 (1918).
7. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 48 (1916).
8. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 261 (1905).
9. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 14 (1910).
10. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **2**, 777 (1896).
11. *Centr. Bakteriol. Parasitenk.*, Abt. 1, **34**, 737 (1903).
12. *Ia. Agr. Expt. Sta. Research Bull.* 99 (1926).
13. *Proc. Soc. Appl. Bacteriol.*, 47 (1945).
14. *Proc. World's Dairy Congr., 11th Congr.*, **2**, 111 (1937).
15. *Scot. J. Agr.*, **17**, 293 (1934).
16. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 559 (1949).
17. *J. Dairy Sci.*, **14**, 457 (1931).
18. *J. Dairy Research*, **15**, 89 (1947).
19. *J. Dairy Sci.*, **37**, 1316 (1954).
20. *Ia. Agr. Expt. Sta. Research Bull.* 74 (1923).
21. *J. Dairy Sci.*, **14**, 40 (1931).
22. *J. Dairy Sci.*, **34**, 374 (1951).
23. *J. Dairy Sci.*, **26**, 371 (1943).
24. *Intern. Dairy Congr., Proc. 12th Congr.*, **5**, 19 (1949).
25. *J. Dairy Sci.*, **34**, 446 (1951).
26. *J. Dairy Sci.*, **36**, 1104 (1953).
27. *J. Dairy Sci.*, **36**, 1212 (1953).
28. *J. Dairy Sci.*, **37**, 790 (1954).
29. *J. Dairy Sci.*, **37**, 795 (1954).
30. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 74 (1919).
31. *J. Agr. Research*, **13**, 235 (1918).
32. *Ia. Agr. Expt. Sta. Research Bull.* 80 (1923).
33. *Mich. Agr. Expt. Sta. Spec. Bull.* 42 (1908).
34. *Mich. Agr. Expt. Sta. Tech. Bull.* 15 (1912).
35. *J. Dairy Research*, **2**, 164 (1931).
36. *J. Bacteriol.*, **16**, 321 (1928).
37. *J. Bacteriol.*, **16**, 211 (1928).
38. *J. Dairy Sci.*, **34**, 1136 (1951).
39. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 59 (1909).
40. *Johns Hopkins Hosp. Bull.* 26, 386 (1915).
41. *J. Bacteriol.*, **30**, 639 (1935).
42. *J. Dairy Research*, **15**, 409 (1948).
43. *J. Dairy Sci.*, **38**, 1184 (1955).
44. *J. Dairy Research*, **16**, 152 (1949).
45. *Ia. Agr. Expt. Sta. Research Bull.* 206 (1936).
46. *Wis. Agr. Expt. Sta. Research Bull.* 6 (1909).
47. *J. Infectious Diseases*, **6**, 304 (1909).
48. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 412 (1915).
49. *Landwirtsch. Jahrb. Schweiz*, **5**, 16 (1891).
50. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **5**, 241 (1899).
51. *J. Bacteriol.*, **13**, 60 (1927).
52. *Jahrb. Kinderhielk.*, **52**, 38 (1900).
53. *Rev. méd. Suisse romande*, **25**, 714 (1905).
54. *J. Bacteriol.*, **52**, 657 (1946).
55. *J. Dairy Research*, **20**, 36 (1953).
56. *J. Gen. Microbiol.*, **12**, 123 and 133 (1955).
57. *Kan. Agr. Expt. Sta. Tech. Bull.* 2 (1916).
58. *J. Bacteriol.*, **1**, 445 (1916).
59. *J. Bacteriol.*, **6**, 45 (1921).
60. *J. Bacteriol.*, **11**, 115 (1926).
61. *J. Bacteriol.*, **23**, 301 (1932).
62. *Ia. State Coll. J. Sci.*, **6**, 89 (1931).
63. *J. Bacteriol.*, **3**, 293 (1918).
64. *Ia. Agr. Expt. Sta. Research Bull.* 61 (1920).

65. *Brauwelt,* **11,** 170 (1946).
66. *J. Infectious Diseases,* **15,** 100 (1914).
67. *J. Infectious Diseases,* **17,** 137 (1915).
68. *J. Dairy Research,* **6,** 383 (1935).
69. *U. S. Dept. Agr. Bull.* 739 (1918).
70. *J. Dairy Sci.,* **2,** 460 (1919).
71. *J. Dairy Research,* **5,** 15 (1933).
72. *Intern. Assoc. Dairy and Milk Inspectors, 24th Ann. Rept.,* 116 (1935).
73. *Ia. Eng. Expt. Sta. Bull.* 62 (1921).
74. *J. Dairy Sci.,* **2,** 108 (1919).
75. *J. Milk and Food Technol.,* **13,** 350 (1950).
76. *J. Dairy Research,* **6,** 148 (1935).
77. *J. Infectious Diseases,* **3,** 173 (1906).
78. *J. Bacteriol.,* **25,** 461 (1933).
79. *J. Dairy Sci.,* **34,** 11 (1951).
80. *Proc. Soc. Appl. Bacteriol.,* 17 (1949).
81. *Milch-Ztg.,* **18,** 941 (1889).
82. *Ia. State Coll. J. Sci.,* **10,** 261 (1936).
83. *Ia. Agr. Expt. Sta. Research Bull.* 22 (1915)
84. *Appl. Microbiol.,* **2,** 95 (1954).
85. *J. Bacteriol.,* **23,** 59 (1932).
86. *J. Dairy Sci.,* **3,** 291 (1920).
87. *J. Dept. Agr. (Ireland),* **38,** 68 (1941).
88. *J. Milk Technol.,* **6,** 263 (1943).
89. *Proc. Soc. Agr. Bacteriologists* 34 (1943).
90. *Proc. Soc. Appl. Bacteriol.,* 91 (1946).
91. *Appl. Microbiol.,* **2,** 97 (1954).
92. *Food Research,* **19,** 250 (1954).
93. *Food Research,* **19,** 254 (1954).
94. *Ill. Agr. Expt. Sta. Bull.* 228 (1920).
95. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 165 (1899).
96. *Cornell Vet.,* **21,** 109 (1931).
97. *J. Dairy Sci.,* **3,** 502 (1920).
98. *J. Dairy Sci.,* **14,** 27 (1931).
99. *Dairy Inds.,* **13,** 854 (1948).
100. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 631 (1933).
101. *J. Dairy Sci.,* **6,** 616 (1923).
102. *Food Research,* **19,** 246 (1954).
103. *J. Dairy Sci.,* **6,** 127 (1923).
104. *J. Dairy Sci.,* **8,** 370 (1925).
105. *J. Bacteriol.,* **1,** 273 (1916).
106. *J. Dairy Sci.,* **11,** 89 (1928).
107. *J. Bacteriol.,* **59,** 645 (1950).
108. *J. Bacteriol.,* **27,** 487 (1934).
109. *Ia. Agr. Expt. Sta. Research Bull.* 225 (1937).
110. *Centr. Bakteriol. Parasitenk.,* Abt. 2, **20,** 474 (1908).
111. *Intern. Assoc. Milk Dealers, 13th Ann. Conv., Lab. Sec.,* 19 (1937).
112. *J. Dairy Research,* **16,** 46 (1949).
113. *J. Dairy Sci.,* **5,** 485 (1922).
114. *Milk Plant Monthly,* **20** (1), 52 (1931).
115. *Ia. State Coll. J. Sci.,* **9,** 125 (1934).
116. *J. Dairy Sci.,* **26,** 587 (1943).
117. *Ia. Agr. Expt. Sta. Research Bull.* 15 (1914).
118. *Ia. Agr. Expt. Sta. Research Bull.* 20 (1915).
119. *J. Dairy Sci.,* **31,** 139 (1948).
120. *J. Dairy Sci.,* **30,** 263 (1947).
121. *Milchwissenschaft,* **2,** 382 (1947).
122. *J. Dairy Sci.,* **34,** 615 (1951).
123. *Can. J. Technol.,* **31,** 85 (1953).
124. *Can. J. Technol.,* **31,** 92 (1953).
125. *Milchwissenschaft,* **4,** 147 (1949).
126. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 82 (1906).
127. *J. Dairy Sci.,* **20,** 447 (1937).
128. *J. Dairy Sci.,* **35,** 298 (1952).
129. *J. Dairy Sci.,* **35,** 455 (1952).
130. *Proc. Soc. Exptl. Biol. Med.,* **43,** 148 (1940).
131. *Mo. Agr. Expt. Sta. Research Bull.* 388 (1945).
132. *Food Research,* **2,** 505 (1937).
133. *Mikrobiologiya,* **16,** 51 (1947).
134. *Ia. Agr. Expt. Sta. Research Bull.* 29 (1916).
135. *J. Dairy Sci.,* **14,** 276 (1931).
136. *J. Dairy Sci.,* **27,** 753 (1944).

Bacteriology of Milk Production

4

During production and handling of milk on farms, micro-organisms ordinarily are added to it from a variety of sources. The numbers contained when milk leaves the stable vary from a few thousand per milliliter, or even less than 1,000 with well-controlled methods of production, to 500,000, or more, when unsatisfactory conditions prevail. Temperature and period of holding largely determine the extent of the growth that occurs later, but the contamination provides the initial flora and thus assumes an importance beyond the actual number of organisms added.

Because of differences in the general character and importance of the organisms involved, the contamination of milk on farms logically is divided into (1) contamination from interior of the udder, and (2) contamination from external sources.

CONTAMINATION FROM INTERIOR OF THE UDDER

At one time it commonly was believed that milk, especially that drawn toward the end of the milking, could be obtained in a sterile condition. The idea was not the result of careful investigation but was based largely on trials in which a few milliliters of apparently sterile milk were collected and on the fact that milk obtained asep-

tically often shows little tendency to undergo rapid changes. Both Moore [1] and Ward [2,3] reported results which make the idea untenable; they examined udders of cows killed after being milked dry and found that organisms could be obtained regularly from the glandular tissue. The types recovered seemed to be identical with certain of those found in aseptically-drawn milk. Ward examined 19 udders and noted that the lactiferous ducts harbored bacteria throughout their whole extent. He concluded: "Milk, when secreted by the glands of the healthy udder, is sterile. It may, however, immediately become contaminated by the bacteria which are normally present in the smaller milk ducts of the udder."

It is now generally agreed that milk contains bacteria at the time it is drawn as a result of contamination in the milk ducts and cistern. Growth of organisms in these passages during the periods between milkings provides the supply that makes possible continued contamination of the milk.

Numbers of Bacteria in Milk Drawn Aseptically. The organisms in milk drawn aseptically are difficult to estimate accurately; the tendency to form clumps suggests unusually large errors with the plate method, and the numbers ordinarily are too small for the usual microscopic counts. The plate method has been used most often.

Plate counts on milk drawn aseptically from normal udders commonly are low, often a few hundred per milliliter although some counts are higher. Average values from various early studies are as follows:

Average Plate Counts on Milk Drawn Aseptically

Number of Samples	Time of Sampling	Average Number of Bacteria per Milliliter	Reference
316	Strippings	518	Harding and Wilson [4]
730		420	Harding and Wilson [4]
184		320	Harding and Wilson [4]
160	⅓ milk drawn	1,541	Copeland and Olson [5]
45	Strippings	964	Breed [6]
284		658	Wayne and Macy [7]

In Utah, Jones and Stevens [8] selected 10 cows that consistently gave negative tests for tuberculosis, brucellosis, and mastitis and never had shown any symptoms of infectious mastitis. Over a 4-month period, milk from each quarter of the udders of the 10 cows gave an average of 239 bacteria per ml, with a minimum of 49 and a maximum of 511.

Shah and Laxminarayana [9] found that samples of milk collected aseptically from 50 cows had counts from 100 to 9,500 per ml.

There is considerable variation in plate counts on milk from herds and also from individual cows. Dorner [10] examined 993 samples from 132 cows in 6 herds; the herd counts ranged from 530 to 4,390 per ml and averaged 2,775. Jensen and Patch [11] reported that, from day to day, the milk of one cow varied in count from 1,000 to 16,500 per ml and that from another cow ranged from 2,000 to 23,000.

The ratio between plate and microscopic counts on aseptically-drawn milk is usually wide. Thornton and Strynadka [12] examined aseptically-drawn milk with the microscopic method, using 1,000 fields for a determination. Each sample represented about equal amounts of milk from each milking quarter taken after about half the milk had been drawn; 88 samples of macroscopically normal milk were studied. With the 31 samples having leucocyte counts not over 500,000 per ml, the microscopic counts averaged 34,980 per ml, with a minimum of 3,000 and a maximum of 231,600; 10 samples gave counts under 10,000, and 2 gave counts over 100,000. On 17 of these samples the plate counts averaged 918 per ml, with a maximum of 4,600. With the 57 samples having leucocyte counts over 500,000 per ml, the microscopic counts averaged 204,514 per ml, with a minimum of 3,600 and a maximum of 2,562,000; 6 of the samples had counts under 10,000, and 4 had counts over 1,000,000. Thirty-two of the 57 samples had an average plate count of 36,625 per ml, with a minimum of 150 and a maximum of 511,000. Rods were not identified in the samples. With the low and the high leucocyte samples, respectively, single cocci were found in 35.5 and 15.8%; groups no larger than diplococci in 38.7 and 24.6%; streptococci in 16.6 and 42.1%; and nonchain groups in 22.6 and 26.3%.

Excessive Counts on Milk Drawn Aseptically. Some animals with apparently normal udders give milk that commonly contains unusually large numbers of bacteria. Two such cows were studied by Hastings and Hoffmann; [13] 61 samples from one cow (covering 15 months and parts of 2 lactation periods) had plate counts from 1,700 to 305,000 per ml (average 30,700), and 31 samples from another cow, excluding 2 very high counts, had values from 2,500 to 154,000 (average 38,800). Counts on 27 samples from a cow giving milk with more normal numbers of bacteria were minimum 50 per ml, maximum 4,250, and average 800.

Copeland and Olson [5] made numerous counts, during one lactation period, on milk from a cow with an apparently normal udder and

found that 40 exceeded 50,000 per ml and 17 exceeded 100,000; the highest count obtained was 347,000.

Cows giving milk containing excessive numbers of bacteria undoubtedly are of significance in the production of milk that must comply with rigid bacterial standards. In some instances failure of certified milk to meet the bacteriological requirements has been due to a few cows giving exceptionally high-count milk, and exclusion of this milk has brought the numbers of organisms within the limits set. Such cows appear to have normal udders and can be detected only by running counts on milk from individual animals in the herd.

Distribution of Organisms throughout the Milking. There is considerable variation in the numbers of organisms per milliliter in different portions of the milking, the foremilk quite regularly containing the largest number. In a series of comparisons, von Freudenreich [14] found the foremilk averaged 6,505 per ml, the middle milk 1,341, and the strippings 769; the foremilk did not always show the highest count in the comparisons.

Harding and Wilson [4] examined the milk from 5 cows on 6 successive days, the milk from each quarter being studied separately, and found the general average as follows: foremilk 458 per ml, middle milk 187, and last milk 274. Copeland and Olson [5] determined the numbers of bacteria in the foremilk, middle milk, and strippings from each quarter of 8 cows. Omitting the counts from one infected quarter, the foremilk averaged 5,989 per ml, the middle milk 557, and the strippings 415. The bacterial content of foremilk was studied in detail by Faber.[15] The first stream from each quarter of the udder was drawn into a sterile bottle and the procedure then repeated with other streams. The average bacterial counts obtained in 100 series of analyses were first stream 1,275 per ml, second stream 927, third stream 705, fourth stream 647, fifth stream 551, and eleventh stream 449. The first 5 streams had an average count of 821 per ml; the middle milk had an average count of 449.

The comparatively high bacterial content of foremilk is due to this portion of the milk washing out most of the easily removable organisms present in the milk passages. Contamination from these sources persists during the remainder of the milking through the gradual dislodging of organisms but is less per milliliter of milk. When the strippings have a higher bacterial content than the middle milk, manipulation of the udder incident to the stripping may be a factor.

Effect of Elimination of Foremilk. Since the foremilk contains more bacteria per milliliter than the remainder of the milking, elimi-

nation of the foremilk has been suggested as a means of reducing contamination from the interior of the udder. Early results indicated a reduction in count of only about 4%. With sanitary conditions of production, Lochhead [16] found that discarding the foremilk reduced the count from 3,770 to 3,200 per ml; with insanitary conditions the reduction was from 397,600 to 393,800.

The small reduction in bacterial count due to discarding the foremilk is in agreement with the small volume of this portion of the milking. Elimination of the foremilk may be of value when the general conditions of production are excellent and very low bacterial counts are desired; it is of no significance with unsatisfactory conditions of production. Another point to be considered is the flavor of the foremilk; frequently, this is unsatisfactory and discarding the foremilk may be an advantage if milk with exceptionally fine flavor is desired. Rejection of the foremilk involves little fat loss because this portion of the milking is comparatively low in fat. It should not be drawn on the floor since its fermentation there may cause undesirable odors in the stable.

Numbers of Bacteria in Milk from Different Quarters. Data obtained by various investigators show that there may be considerable variation in bacterial counts on milk from the different quarters of an udder. For example, Jensen and Patch [11] noted variations from 10 to 900 per ml with one cow and from 150 to 3,400 with another. Since the bacterial count on the entire milking is a composite of the counts on milk from the different quarters, unusually low or high counts on milk from one quarter are counterbalanced by counts on milk from the other quarters. Most investigators have noted no regularity in the quarters supplying milk showing either relatively low or high counts. Differences also have been reported in the types of organisms from the different quarters.

Influence of Period of Lactation. The period of lactation apparently has no marked influence on the bacterial content of aseptically-drawn milk.[4,5] Colostrum milk often has a relatively low count,[7,17] but high counts also have been reported.[5]

Influence of Various Factors. Age of the cow, character of the feed, and similar factors apparently have no definite influence on the bacterial content of aseptically-drawn milk. In studies on the effect of various methods of drying up cows on the bacterial content of milk, Wayne and Macy [7] found that intermittent and incomplete milking usually resulted in somewhat higher bacterial counts during such periods, and that immediately following periods of suspended milking

the bacterial counts were slightly higher. None of the milking procedures used in drying up the animals appeared to have any marked effect on the bacterial content of the milk in subsequent lactation periods.

Influence of Various Treatments on Bacteria in the Udder. Atwood and Giddings [17] attempted to reduce the contamination of milk drawn under controlled conditions. After the udder had been thoroughly stripped, the end of the teat was dried with a sterile towel and moistened with 25% lysol; this was wiped off, carbolated vaseline rubbed on, and the vaseline protected with adhesive tape. Counts obtained on the milk after such treatment did not differ significantly from those obtained on milk produced normally, which indicates that contamination through the teat canal has little influence on the number of organisms coming from the interior of the udder. Injection into the udder of materials intended to destroy or inhibit bacteria caused inflammations.

Hoffmann [13] injected udders with physiological salt solution, 2% boric acid solution, or distilled water and found that such inert materials produced rapid and marked leucocytosis. In some instances an increased leucocyte count was accompanied by a decreased bacterial count. This may have been due to phagocytic action since microscopic preparations showed many leucocytes containing from 5 to 25 bacteria while leucocytes from uninjected quarters showed no such activity.

Wallis *et al.*[18] reported that sealing the teats with collodion between milkings seemed to have no noticeable effect in reducing bacterial counts on milk or preventing relatively wide fluctuations in the counts.

Types of Organisms in Milk Drawn Aseptically. The organisms in milk drawn aseptically belong to certain more or less definite types. To multiply in the udder, an organism must have the ability to grow in contact with the tissues there, and this is lacking in many common species. Some of the species that develop rapidly in milk after it is drawn, die quickly when introduced into the udder. Ward [2] injected *Ser. marcescens* through the teat canal and, although an infection was set up, only a few colonies developed on plates poured after 24 hr; none developed on plates poured on the sixth day. Russell and Hastings [19] injected four species into the udder, the common lactic acid organism, an acid-producing liquefying form, *Ser. marcescens*, and a yellow liquefying coccus frequently in foremilk; in all cases the numbers of the organisms recovered on plates became smaller as the period of exposure in the udder increased. In most instances injection of an

organism resulted in the milk becoming more or less abnormal and sometimes the udder was inflamed. Copeland and Olson [5] injected *A. aerogenes* into one quarter and, although this became swollen and enlarged, the organism survived only a few days.

Micrococci are particularly prominent among the organisms isolated from aseptically-drawn milk and also are conspicuous among the organisms obtained from udder tissues. Evans [20] studied 192 samples of milk from 161 cows, representing 5 different dairies, and found micrococci in 113 (58.9%); the highest number was 80,000 per ml. Breed [6] found 12 species of micrococci in strippings drawn under controlled conditions. Jacquet *et al.*[21] isolated 48 strains of micrococci from the udders of healthy cows and identified various species. All of the strains were aerobic and survived pasteurization.

The udder micrococci often produce colored colonies on agar, especially yellow or orange; chromogenesis commonly is more conspicuous on plates incubated at 69.8 F (21 C) than at 98.6 F (37 C). In general, the organisms produce changes in milk comparatively slowly and thus are of little importance in the spoilage of milk.

Streptococci also are present in milk from normal udders. Sherman and Hastings [22] found them in 0.01 ml of the mixed milk from 10 of 12 herds and in 38.6% of the individual samples from 88 cows in 4 herds. Evans [20] obtained long-chained streptococci which were not *S. lactis* from 29 (15.1%) of 192 samples studied, the highest number being 264,000 per ml.

By means of special media, especially agar to which blood serum has been added, rod-shaped bacteria have been isolated from milk drawn aseptically. Evans [20] recovered them from a considerable percentage of the samples examined, sometimes in large numbers. The organisms,[23] which grew rapidly in milk, were of different types, and one is of special interest since, with high initial contamination of cream, it hydrolyzed butterfat and produced a disagreeable flavor and odor in 24 hr.

Knowles [24] plated samples of foremilk from 4 healthy cows on litmus lactose agar and incubated the plates at 86 F (30 C) for 40 hr; counts per milliliter were 40, 65, 3,220, and 145. The number of liquefiers per milliliter on litmus lactose gelatin plates incubated at 71.6 F (22 C) for 40 hr were 0, 7, 1,200, and 6. They included *Micrococcus caseolyticus, M. freudenreichii,* and *S. liquefaciens.* The author concluded the foremilk may contribute to cheese acidoproteolytic organisms which aid in the ripening process.

Occasionally, organisms other than the usual udder types are present

in milk as a result of contamination in the udder. In some cases they are related to abnormal udder conditions; in other instances they are organisms that have penetrated the teat canal or been brought to the udder through the blood stream and probably will soon disappear from the udder because of their inability to survive the conditions there. Rarely, organisms that are present in milk in considerable numbers as it leaves the udder may be involved in rapid deterioration of the product.

Pathogens in Milk Drawn Aseptically. As a result of an infection in the producing animal, milk sometimes contains pathogens at the time it leaves the udder. The organisms causing tuberculosis, brucellosis, and certain udder infections are the most important of these (see Chapter 9).

CONTAMINATION FROM EXTERNAL SOURCES

Bacteria from external sources are added to milk during the milking process. Two methods of milking are in use, hand milking and machine milking, and they present somewhat different bacteriological problems. With either type of milking, milk houses frequently are used and often are required by regulations. They include facilities for cooling the milk, caring for equipment, etc., and should be distinctly separate from the stable to avoid stable odors.

Hand Milking

With hand milking the external sources from which micro-organisms are added to milk include (1) stable air, (2) coat of the animal, (3) milk equipment, (4) milker, and (5) miscellaneous sources.

1. Stable Air

Stable air may contain dust in considerable quantities, and this has led to the assumption that air is an important factor in the bacterial contamination of milk. However, investigations have shown that, although some organisms enter milk from this source, the numbers ordinarily are not large; it is probable that the desiccation and light to which organisms carried by dust are subjected destroy many of them so that material falling into milk from the air adds comparatively few living organisms. Dusty air definitely tends to increase the amount of sediment in milk.

Methods of Measuring Contamination. The extent of contamination of milk from the air has been investigated in various ways.

One of the simplest methods is to expose agar plates of a known area for a given period in the stable to be studied and then calculate, from the colonies developing on incubation, the number of organisms that would fall into a pail with a certain opening during the milking period. The method is open to criticism since the organisms present on a dust particle may be so close together that several develop into a single colony, and it also is probable that some organisms are held away from the surface of the agar and do not develop at all. Sterile milk or water can be exposed in a pail and the organisms falling into it from the air determined by making counts. Variations in air conditions can be shown by determining the numbers of organisms in the air, but such data give no direct information on the numbers that would be added to milk.

The effect, on the bacterial content of milk, of increasing the dust in the air by some such process as feeding can be investigated by milking half of a group of cows when the air is comparatively free of dust and the other half after carrying out the operation being studied. The two lots of cows are alternated in different comparisons to compensate, as far as possible, for variations in contamination from the interiors of the udders.

Influence of Stable Conditions. Stocking [25] studied the influence of feeding hay and grain just before milking on the bacterial content of milk, using 10 cows in 6 comparisons and determining the organisms by the plate method. When the cows were milked before feeding, the average number of bacteria per milliliter was 2,096 and when milked after feeding the number was 3,506. With various stable conditions, Lochhead [16] determined the numbers of bacteria falling per minute into an 11-in. pail and then calculated the contamination of milk on the basis of 10 lb drawn in 8 min. With no sweeping or handling of hay 1 hr before milking, the calculated value was 22 per ml; with feeding hay 10 min before milking, it was 45; and, with cleaning and sweeping 10 min before milking, it was 62.

Bacterial Content of Stable Air. Ruehle and Kulp [26] investigated the bacterial content of stable air and its effect on the bacterial content of milk using aeroscopes and an "artificial udder" from which sterile water could be "milked." When the cows were in the stable, the bacterial content of the air usually was between 50 and 100 per liter with a maximum of 825. Sterile water "milked" under these conditions had an average bacterial content of 12 per ml, the highest count being 73. When a heavy dust was raised in the stable loft by sweeping up material from the floor, the bacterial content of the air

was from 1,000 to 2,000 per liter and sterile water "milked" under these conditions had an average bacterial content of 47.6 per ml. By raising dust continuously during the test, the bacterial content of the air increased to between 2,500 and 10,000 per liter and the average count of the water "milked" was 604 per ml.

Reducing Contamination from Stable Air. Reduction of contamination from stable air involves primarily a logical selection of the time for such operations as brushing, feeding, and bedding the animals so that the air will be reasonably free of dust during the milking. It is easier to keep the stable air in a satisfactory condition when the floors, walls, and ceiling are clean than when they are covered with dust and dirt.

Types of Organisms from Air. Because of the severe conditions existing in the air, most of the organisms coming from this source must be comparatively resistant. They include spore-forming bacteria, both aerobic and anaerobic, certain cocci, especially micrococci, and various yeasts and molds. Organisms that have little resistance sometimes are encountered; presumably, they have been in the air only a short time.

2. Coat of the Animal

During the milking process, material falls from the coat of the cow, and a portion of it gets into the milk. It may include manure, bedding, soil, epithelial cells, etc.; it carries organisms, often in large numbers, and these are readily distributed through the milk. A considerable part of the sediment in milk often comes from the coats of cows although other sources also are involved.

Amount of Dirt in Milk. The amount of dirt in milk can be determined on a comparative basis by use of cotton discs, or on an actual weight basis by collecting it through sedimentation and/or centrifuging; none of the methods recovers all the dirt present. Harding and Prucha [27] found that, when the cows were unusually dirty and were milked into an open pail, dirt in the unstrained milk amounted to 10.8 mg per qt, whereas with methods comparable to those used in the better dairies the amount was about 2.5 mg. Under the former conditions the increase in bacterial content of the milk due to the dirt was about 17,000 per ml. The bacterial content of dirt from the coat of a clean cow was found to be 17,814,000 per g; that from the coats of 4 dirty cows varied from 184,300,000 to 4,592,000,000. Considerable variation in the bacterial content is to be expected because of differences in the nature of the dirt.

Bacteria in Cow Manure. Prucha *et al.*[28] collected 27 samples of fresh cow feces, held them at about 75 F and a relative humidity between 20 and 40, and determined the numbers of bacteria by the plate method at intervals during 3 weeks. The bacterial content of the fresh feces ranged from 434,000 to 73,200,000 per g of dry matter and averaged 8,486,000; the moisture content varied from 78.4 to 87.6% and averaged 83.6. In all the samples there was multiplication of the bacteria present. The rate of increase was very rapid during the first 2 days, after which it became slower but continued for several days until a maximum was reached. A slow decrease then began. The maximum number in nearly every sample reached several billion per g of dry matter; in 6 it was between 10 and 11 billion and in only 1 was it <1 billion. Many of the organisms present were gas formers.

Allen,[29] using the plate method, found that 50 samples of fresh cow feces contained 160,000 to 634,000,000 organisms per g, and 49 samples dried on porous plates at 98.6 F for 48 hr contained 500,000,000 to 16,800,000,000. Representative colonies from plates poured with the fresh feces were inoculated into litmus milk and the changes produced showed that a variety of organisms was present.

Solubility of Cow Manure in Milk. Taylor[30] investigated the solubility of cow manure in milk and summarized the results as follows:

Average Solubility of Cow Manure in Milk

	Moisture in Manure (%)	Solids in Manure (%)	
		Soluble in Milk	Insoluble in Milk
Fresh manure	82.76	0.96	16.25
Air-dried manure	5.74	5.22	89.04

Manure was found to be less soluble in milk than in water. The results also indicated that 91% of the manure present in bottled milk will be visible at the bottom of the bottle.

Effect of Dirt on Flavor of Milk. Marquardt[31] noted that the flavor of milk improved with an increase in the percentage of clean milk included. The flavor of clean milk deteriorated more slowly than that of unclean milk when held 4 to 6 hr at 70 F.

Detection of Manure Fragments in Milk. Bits of plant tissue may be in milk as a result of contamination with either dust and dirt or manure. From the standpoint of milk control, the latter source is much the more objectionable. Staining procedures[32] have been de-

veloped for detection of manure fragments in dairy products so that contamination with manure cannot be ascribed to other materials.

Species of Organisms from Coats of Cows. The species of organisms entering milk from the coats of the cows are, in general, those found in manure, soil, feeds, and bedding. Many of them are very objectionable because of the rapid changes which they bring about in milk.

Influence of Clean Cows on Bacterial Count. Control of contamination from the coats of the cows requires that the animals be kept reasonably clean. This involves frequent brushing and, on many farms, washing or wiping the udders and flanks; occasionally, more complete washing of the animals is practiced. Method of bedding and type of stanchion are important factors in preventing coats of the animals from accumulating manure and dirt.

Stocking [25] studied the effect of wiping the udders and flanks with a damp cloth on the bacterial content of milk, using 2 groups of 5 cows each and milking 1 group with such wiping and the other without, the groups being alternated. A small top pail was used and the milk from the 5 cows in a group was mixed before sampling. The average plate counts of the milk were 7,058 per ml when the udders and flanks were not wiped and 716 when they were wiped.

Data presented by Ayers *et al.*[33] show the influence of cleaning the cows and floors on the bacterial content of milk; one effect of clean floors was to keep the animals clean. Samples from the dirty animals were obtained from April 11 to May 6, inclusive, and those from the clean animals from June 5 to 13, inclusive. The summarized results are as follows:

Effect of Condition of the Cows on Bacterial Content of Milk

	Cows and Floor Dirty, Manure Removed Weekly, Utensils Sterile		Cows and Floor Clean, Manure Removed Daily, Udders and Teats Washed, Utensils Sterile	
	Number of Samples	Average Number Bacteria per Milliliter	Number of Samples	Average Number Bacteria per Milliliter
Open pail	41	86,212	15	4,947
Small top pail	41	24,439	15	2,667

The reduction due to cleaning of the animals and washing of the udders and teats was pronounced.

Small Top Pails. Small top or sanitary milk pails are intended to reduce the area through which material can fall into the milk and, in this way, decrease contamination from the coat of the animal and the air. They are very helpful in producing clean milk and are employed widely. Simple construction is advisable so that the pails can be washed and used without inconvenience.

Ayers *et al.*[33] compared the counts obtained with open and small top pails under different conditions of production, with results as follows:

Influence of Small Top Pails on Bacterial Content of Milk

Conditions of Production	Open Pails		Small Top Pails	
	Number of Samples	Average Number of Bacteria per Milliliter	Number of Samples	Average Number of Bacteria per Milliliter
Cows and floor dirty, manure removed weekly, utensils not sterile	26	497,653	28	368,214
Cows and floor dirty, manure removed weekly, utensils sterile	36	22,677	36	17,027
Cows and floor dirty, manure removed twice a week, udders and teats washed, utensils sterile	18	6,166	23	2,886
Cows and floor dirty, manure removed weekly, utensils sterile	41	86,212	41	24,439
Cows and floor dirty, manure removed weekly, utensils not sterile	36	153,905	36	114,497
Cows and floor clean, manure removed daily, udders and teats washed, utensils sterile	15	4,947	15	2,667

Under all the conditions studied, milk drawn in small top pails had a lower bacterial count than that drawn in open pails and, commonly, the difference was pronounced.

The following data, reported by Lochhead,[16] also show the effect of small top pails in reducing the bacterial count of milk.

Influence of Small Top Pails in Reducing Bacterial Content of Milk

	Bacteria per Milliliter of Milk		
	Open Pail	Small Top Pail	Decrease
Generally sanitary, careful methods	4,560	3,200	1,360
Pails clean, animals and stable clean, milker careless	10,490	9,150	1,340
Pails clean, cows milked under careful conditions, stable clean, animals not regularly cleaned	37,670	12,000	25,670
Generally careless conditions	397,600	192,500	205,100

Reduction in the bacterial content of milk as a result of use of small top pails is extremely variable and depends on such factors as condition of the coat of the cow and amount of dust in the air. Under unsatisfactory conditions of production the number of organisms kept out of the milk per milliliter is larger than under good conditions, but in both cases reduction percentage-wise is considerable.

Influence of Clipping Udders, etc. Hird *et al.*[34] found that clipping the thighs, flanks, udder, and tail (except the switch) of cows tended to lower the plate count of milk, whether the milking was by hand or machine. The average counts obtained by hand from clipped and unclipped cows were 1,643 and 2,996 per ml and by machine they were 3,042 and 3,458, respectively. The advantage of clipping was statistically significant with hand milking. Clipping of cows caused no measurable difference in the amount of extraneous matter in milk with either hand or machine milking.

3. Milk Equipment

Unless given adequate care, such equipment as pails, cans, coolers, etc., with which milk comes in contact on farms, is a serious source of bacterial contamination and may be the most important one.

Methods of Measuring Contamination. A common method of measuring contamination from a milk container is to add a known volume of sterile nontoxic tap water, buffered distilled water, or comparable material, suspend the organisms in it as completely as possible by shaking or with a sterile brush, determine the bacteria per milliliter of the rinse material, and calculate the number of organisms in the container.[35] If preferred, the result can be calculated to the number of organisms that would be added per milliliter if the container were filled with milk. The general procedure has been modified in several ways, depending on the container and other factors. Results are low because of the difficulty in dislodging organisms and breaking up clumps. Organisms from equipment on which growth has occurred often are in clumps.

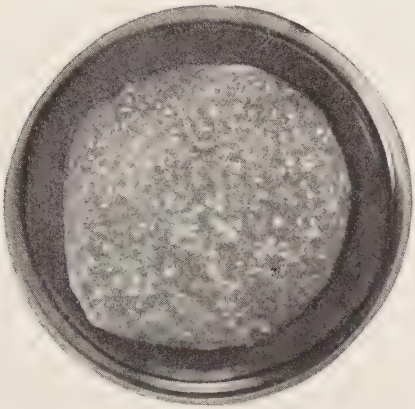
A culture dish spot plate can be filled with agar, pressed lightly against a surface to be examined, and then held in a sterile petri dish. After incubation the colonies are counted. Swab procedures have been devised in which a swab is rubbed over an area to pick up organisms.[35] The swab then is treated in various ways to disperse the organisms in a dilution blank and the blank plated. Swab test methods are proving useful in examination of equipment in various food processing plants. Jamieson and Chen[36] swabbed the surfaces

of equipment with sterile cotton swabs, which were moistened with sterile water for use on dry surfaces, and then smeared each swab on a suitable medium in slanted oval test tubes or in flat bottles with caps. Reliability of the test was substantiated by statistical studies. The test can be carried out readily in a plant with transported equipment.

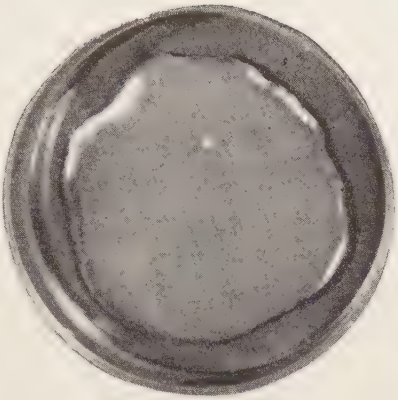
Olson and Hammer [37] suggested an agar disc method for studying contamination from utensils and dairy plant equipment. The medium is made with 2.5% air-dried agar so the disc will be strong enough to permit handling; the other ingredients can be those needed for any desired medium. After liquefying and cooling the medium to about 109.4 F (43 C), a small amount is poured on the surface to be studied and allowed to solidify. The disc thus formed is picked up with a sterile spatula and tipped into a sterile petri dish so that the portion in contact with the surface is toward the top. After incubation under the conditions desired, the colonies developing on a measured area (usually 20 to 30 sq cm) are counted and the results expressed as the number per square centimeter. With heavily seeded discs, approximate counts or estimates are necessary. The method is useful primarily for comparative purposes. It gives a general picture of the surface examined from the standpoint of numbers, distribution, and colony types of the organisms present; such a picture often is more impressive than bacterial counts on rinse water. Discs cannot be prepared in inaccessible places, where proper cleaning is especially difficult, but by tipping equipment various surfaces can be put in such a position that discs can be obtained.

Numbers of Organisms from Equipment. Early studies clearly indicated the importance of equipment as a source of bacteria. Prucha *et al.*[38] found that 170 freshly washed and unsteamed cans contained sufficient bacteria to have added from 197 to 2,557,000 organisms per ml (avg 128,592) if the cans had been filled with milk. Investigation of 91 cans that were washed, rinsed, and steamed at the plant and then covered with their lids and returned to the farms showed that, as they were about to be used, they would have added 23,523 bacteria per ml to the milk; treatment on the farms was not uniform, either as to care of the cans or period of holding. The collective influence of the utensils was studied on 81 cans of milk drawn and handled with sterile utensils in comparison with 117 cans for which the utensils were not sterilized; the average bacterial count on the former was 6,807 per ml and on the latter 285,600.

In the examination of 236 cans under all conditions of washing and



Agar disc from a washed but unsteamed can.



Agar disc from the same can after steaming.

Figure 18. Influence of steaming on organisms in a milk can as shown by the agar disc method. Iowa Agr. Exp. Sta. Bul. 300.

Agar disc from a freshly steamed can that was covered before the moisture could escape.

Agar disc from the same can after standing covered for 24 hr.

Figure 19. Growth of organisms in a moist milk can as shown by the agar disc method. Iowa Agr. Exp. Sta. Bul. 300.

steaming, Smith [39] found that, had the cans been filled with milk, contamination would have varied from 0 to 7,920,000 organisms per ml. In a later study [40] cans were examined at the railway station as they were being sent to shippers; 108 wet cans would have added 547,994 bacteria per ml of milk, with extremes of 52 and 4,332,000, whereas 38 dry cans would have added 1,870 bacteria, with variations from 2 to 19,632. Sixty cans from 4 plants using can washers and air blasts for drying the cans would have added 14 bacteria per ml of milk, with extremes of 0 and 331. In one plant the effect of improved washing equipment on the condition of the cans was clearly evident; with the old method 9 cans would have added 88,500 bacteria per ml of milk, with variations from 23,700 to 178,000, whereas after a can washer with an attached air blast was installed 12 cans would have added 122 bacteria, with variations from 52 to 634.

Other equipment used on farms, such as pails and coolers, often is just as important a source of organisms as cans. It may be even more important since it may be poorly treated at the farms while many plants now return to the farms adequately treated cans which are used as received.

Filtering Milk. Milk on farms commonly is filtered or strained to remove, as completely as possible, material that has fallen into it. Filtering has no significant effect in removing bacteria from milk (see p. 189) and, if the filtering equipment is not in a satisfactory bacteriological condition, it may add bacteria, at least until the organisms have been washed from it.

Growth of Organisms on Equipment. The enormous numbers of organisms on some equipment can be explained only on the basis of growth on it. Extent of the growth is determined by the conditions and species of organisms involved. Water containing traces of milk solids provides a suitable medium for many species, and the temperature at which equipment often is held, especially during warm months, is satisfactory for rapid development. When the washing is carelessly done so that milk solids remain on the equipment in considerable amounts, the number of organisms left after the washing is likely to be high and, moreover, growth is greatly favored by the nutrients thus provided. In addition to causing contamination of dairy products, growth of organisms on equipment is objectionable because of the odors developed, action of bacterial products on the metal, etc.

Species of Organisms from Equipment. The species of organisms added to milk by equipment are varied. Organisms that grow well in milk commonly are prominent among them since, owing to the

presence of milk solids, conditions for growth on equipment are similar to those in milk. This suggests that *S. lactis* may come from equipment and, under average farm conditions, equipment is an important source of it. In certain cases, ropy milk has been caused by contamination from equipment.

Care of Equipment. Washing of equipment should begin with the rinsing away of all adhering milk with cold, or only slightly warmed, water; hot water often causes some of the milk constituents to stick to the metal, and these then are difficult to remove. After rinsing, the equipment should be washed with hot water containing washing reagent. If the wash water is not reasonably clean, it may be an important source of organisms. After proper washing, thorough rinsing again is necessary, and this should be followed by steaming, scalding, or some comparable treatment. Unless a special method of drying is to be used, the metal should be heated so thoroughly that the water remaining after the equipment has been carefully drained will evaporate. Escape of the water vapor is greatly facilitated by leaving pails and cans upright; when they are inverted on racks, the vapor rises and, since it cannot escape readily, is condensed and much of the water remains. Inversion of the dry utensils in a clean atmosphere is an aid in limiting air contamination. Harding *et al.*[41] found that protection of thoroughly steamed milk pails against accidental contamination had a measurable effect in reducing the bacterial content of milk.

Rinsing equipment removes some of the organisms mechanically and then, as the temperature is increased, destruction of the organisms begins.[42] Small amounts of water are cooled quickly when added to equipment and quantities carrying sufficient heat to raise the temperature of the metal properly are necessary.

Equipment is readily heat treated by putting it in a cabinet into which steam can be turned or in a closed metal container in which steam can be generated. Ayers and Mudge[43] considered the possibility of sterilizing utensils with hot air. They found that raising the temperature to 248 F during a 4-min period destroyed a great many bacteria but did not give uniformly low results, whereas an added holding period of 2 min increased the destruction. When a temperature of 284 F was reached in 4 min, there was a high destruction of organisms and holding at this temperature increased it.

The presence of adhering milk solids affects the sterilization of equipment. Heat is transferred relatively slowly to organisms imbedded in the solids. Organisms in fat may be protected additionally.

From the standpoint of food sterilization, Yesair *et al.*[44] noted that moist cocci (staphylococci, streptococci, diplococci, etc.), or those dried over calcium chloride in vacuum, were low in heat resistance under moist conditions. Resistance was higher in moist fat. Resistance in dry fat was exceptionally high and approached that under dry sterilization. The mechanism of fat protection in foods appeared to rest on the localized absence of moisture.

Construction of Equipment. Proper care of equipment is possible only when it is in satisfactory mechanical condition. Rough areas and sharp depressions on a surface make the complete removal of milk solids difficult and thus complicate the washing. Any seams that are present should be well filled with solder so as to avoid points at which milk solids may accumulate. Types of construction in which seams are avoided have a definite bacteriological significance.

Sanitizing Equipment before Use. On many farms equipment is sanitized just before use, even when good washing and sterilizing facilities are available, to be certain that it will not be a source of significant numbers of organisms. The procedure is especially useful if rigid bacterial standards are being met.

Influence of Sterile Equipment on Bacterial Count. Influence of sterile equipment in reducing the bacterial content of milk is illustrated by the findings of Ayers *et al.*[33] in a comparison of sterile pails and cans with those in an unsterile condition. Throughout the trials the floors and cows were dirty, the manure being removed only weekly. Milk representing the unsterile utensils was obtained from July 22 to Aug. 14, inclusive; that representing the sterile utensils was obtained from Sept. 14 to Oct. 7, inclusive. With open pails, the average count when the utensils were not sterile was 497,653 per ml (26 samples), and when the utensils were sterile it was 22,677 (36 samples); with small top pails the corresponding counts were 368,214 (28 samples) and 17,027 (36 samples). The data show that a greatly reduced bacterial count accompanied the sterilization of the utensils, even under the otherwise unsatisfactory conditions of production. Although the counts were lower with the small top pail than with the open pail, sterilization of the utensils was much more important than the type of pail.

Data reported by Lochhead[16] show the great effect of sterilization of small top pails on the bacterial content of milk. When the pails were cleaned with cold water the count was 33,700 per ml; when the pails were rinsed with cold water, washed with hot water, but not

sterilized, it was 14,460; and when the pails were sterilized after washing it was 3,200.

The influence of unsterile equipment on the bacterial content of milk is extremely variable and depends on such factors as care used in the washing, heating incident to the washing, and opportunities for growth of organisms between washing and use. On farms where no attempt is made to give the equipment proper care, large numbers of organisms commonly come from it.

4. Milker

The milker may add organisms to the milk from the hands and clothing, and also by coughing, sneezing, or speaking over the equipment or milk. Organisms from the latter sources are especially objectionable because of the possibility of pathogens being included.

During milking, organisms are dislodged from the hands and often fall into the milk. Dirt may fall into the milk from dirty clothing, especially when a pail is carried so that the top brushes against it. Extent of contamination from the milker is determined largely by the general habits of the individual. A milker should be neat and clean and should appreciate the importance of suitable methods in handling materials intended for human consumption. Clean hands add fewer organisms than dirty ones, and in some dairies interested in production of milk of an exceptionally high sanitary quality the hands are washed before each cow is milked. The milk should not touch the hands since, if it does, it tends to wash off dirt and bacteria that may be present. Clean clothing is very desirable because of the dirt falling from unclean garments. The principal advantage of wearing white suits when working with dairy products is that they quickly show an accumulation of dirt. Such practices as working in a hot, dusty field and then milking without changing the clothing or washing the hands are very objectionable.

5. Miscellaneous Sources

Miscellaneous sources from which milk is contaminated include those that are involved with certain lots of milk but not with others. Examples are flies, contaminated water, visitors contaminating equipment or milk, and dropping of a utensil which then is used without cleaning. In some instances the miscellaneous sources of contamination are of considerable significance.

Flies are responsible for contamination of milk, either indirectly through contaminating equipment or directly by falling into it. In

a series of experiments reported by Esten and Mason [45] in which 414 flies were examined, the numbers of bacteria present on the body of a fly varied from 550 to 6,600,000 and averaged 1,222,570. Armstrong [46] also reported counts of the organisms that could be washed from the bodies of flies; 7 flies caught in clean houses carried from 600 to 75,000 bacteria per fly and averaged 13,986, whereas 6 flies caught in dirty houses carried from 1,050 to 4,000,000 and averaged 1,106,017. Because flies so commonly alight on fecal material, organisms on their bodies include various species, especially *Escherichia-Aerobacter* forms, which rapidly produce changes in milk. When human fecal material is exposed, there is possibility of intestinal pathogens being carried to milk.

Farm water supplies frequently have been found contaminated. Such water may add organisms to equipment washed in it and, if it splashes into the milk when used for cooling, etc., it adds organisms directly to the milk.

Important Factors in Producing Low-count Milk. Factors of importance in producing milk with a low bacterial count are sterile equipment, reasonably clean cows, and small top pails; these form the basis for any contemplated improvement in the sanitary quality of milk. None of the factors involve either an unreasonable effort or prohibitive expense but they do require an understanding of the care necessary in producing a food material, particularly under the conditions existing with milk. Results obtained in widely varying areas indicate that anyone who will use reasonable care can produce milk with a low bacterial count.

Ayers *et al.*[38] studied the effect of sterile, small top pails in reducing the bacterial content of milk on a number of farms during the summer, when the cows were on pasture most of the time and their coats practically free of visible dirt; counts first were obtained for a period on milk produced in the ordinary way and then on that produced with sterile, small top pails. Results are given in the summary on page 137.

North [47] presented data illustrating the effect of sanitary methods in decreasing the bacterial content of milk. First, milk drawn by the usual milkers was studied and, then, milkers experienced in proper methods were brought to the farms. The imported milkers employed the old equipment, except that they used small top pails, and were on the farms not more than 2 hr. Representative counts on the milk obtained by the usual milkers and the experienced milkers, respec-

Influence of Sterile, Small Top Pails on Bacterial Content of Milk When the Cows Were Reasonably Clean

	Open Pails, Utensils Not Sterilized		Small Top Pails, Utensils Sterile	
Farm	Number of Samples	Average Number of Bacteria per Milliliter	Number of Samples	Average Number of Bacteria per Milliliter
1	19	116,384	16	10,637
20	18	15,050	16	4,656
27	19	186,995	16	3,606
29	19	77,095	16	1,962
43	18	34,861	16	2,150

tively, are as follows: Farm *A*, 1,830,000 and 3,300 per ml; farm *B*, 1,520,000 and 3,100; farm *C*, 4,830,000 and 4,600; farm *F*, 3,600,000 and 61,000; farm *G*, 60,000 and 800; farm *J*, 500,000 and 5,600.

Results showing the effect of various factors in reducing the bacterial count of milk were reported by Lochhead,[16] as follows:

Effect of Various Factors in Reducing Bacterial Content of Milk

	Bacteria per Milliliter of Milk
(a) Generally careless conditions throughout	398,000
(b) Careless conditions except that cows were brushed daily, without removing possibility of contact with manure	316,500
(c) Careless conditions except that care was taken to remove manure from contact with cows, cows not brushed	69,200
(d) Careless conditions except that manure was removed and cows brushed daily	48,800
(e) Like (d) but care exercised by the milker	27,600
(f) Like (e) but care given the milk pail	3,200

General Conditions. Certain areas definitely are better adapted than others to producing clean milk with low bacterial counts. In areas having long periods when the animals are in the stable and/or long periods of muddy conditions, it is difficult to keep the coats of the animals clean without special procedures that often are not applied readily, especially to milk intended for manufacturing purposes. As a result there may be considerable sediment in the milk, and the bacterial counts may be relatively high. When conditions are such that the animals are in the stable only for milking and the soil is sand and

gravel, with the rain water quickly being carried away, it is not so difficult to keep the animals clean. Accordingly, there is relatively little sediment in the milk and the bacterial counts are comparatively low, unless there is serious contamination from some such source as equipment. With sand and gravel soil there is less dust in the air and less sediment due to windblown material. Because of the great influence of climatic conditions, control of contamination from the coats of the cows sometimes may require special consideration.

Influence of the Stable on Milk Contamination. At one time the stable was thought to be an important factor in producing milk low in bacteria, and a poor stable often has been given as an excuse for a high count. Investigations have shown, however, that milk of high sanitary quality can be produced in a stable of very poor type.

North [47] studied, for 1 yr, the sanitary quality of the milk produced in 2 barns of very different construction and surroundings, by running counts at least 3 times a week and often daily. In both barns suitable methods were used, but with the old barn, the cow yard, lighting, ventilation, floors, walls, and ceiling were in a very unfavorable condition. The bacterial content of the milk from the old barn averaged 3,102 per ml, with the monthly averages from 331 to 10,200, whereas milk from the sanitary barn averaged 1,097, with the monthly averages from 287 to 3,300.

Although milk of low bacterial content can be produced in a poorly constructed stable, it is easier to obtain milk of good sanitary quality in satisfactory than in unsatisfactory surroundings and, for this reason, regulations covering milk production commonly include requirements with reference to the stable. A suitable building and good equipment are a distinct advantage in the proper handling of any food product and, moreover, the consumer expects all such products to be handled under ideal conditions.

Nutritionally, an increased national consumption of milk is very desirable. It best can be obtained through lower prices, and these are dependent on lower production costs. Fenzau and Van Arsdall [48] reported that preliminary analysis of data from nine experimental farms showed that milk of high quality can be produced with no more than half the labor and half the investment in structures and facilities commonly found on dairy farms in the area. Operators who followed recommended sanitation procedures produced milk with bacterial counts acceptable by grade A standards. Even when milking was done in temporary stalls on an earthen floor in an open shed, without an enclosed milking area, most of the samples were accept-

able. Of 97 samples taken from 3 farms with only these facilities, 85.5% contained <200,000 bacteria per ml and 52.5% contained <20,000. There was no evidence that buildings affected the quality of milk, except as they made it easier to follow good sanitation practices.

Machine Milking

Use of milking machines changes somewhat the relationship of the external sources from which milk is contaminated. With machines, contamination from the air, coat of the animal, and certain other sources is less important than with hand milking and, in general, machines have refined the methods of producing milk. However, unless properly cleaned and sanitized, machines are an important source of organisms.

Early Bacterial Counts on Hand- and Machine-drawn Milk. Some of the early investigations (1897–1913) on the bacteriological quality of machine-drawn milk consisted of comparisons of the counts on milk obtained by hand and by machine. Data in the accompanying table show that the counts commonly were higher with machine milking.

Early Comparisons of Plate Counts on Hand- and Machine-drawn Milk

Number of Trials	Method of Milking	Bacteria per Milliliter of Milk	Investigators
94	Hand	645 to 63,422	Harrison [49]
235	Machine	71,124 to 243,327	
136	Hand	<1,000 (19.8%)	Hastings and Hoffman [50]
		1,000 to 5,000 (44.1%)	
		>5,000 (36.1%)	
150	Machine	<1,000 (22.0%)	
		1,000 to 5,000 (55.3%)	
		>5,000 (22.7%)	
6	Hand	3,200 to 68,900	Edwards [51]
7	Machine	203,000 to 1,208,000	
46	Hand	21,115 (average)	Meek [52]
45	Machine	47,870 (average)	
20	Hand	16,643 (average)	Harding *et al.*[53]
20	Machine	692,542 (average)	
38	Hand	10,739 (average)	Larson *et al.*[54]
38	Machine	509,285 (average)	

From the early studies, it was assumed that the high counts so often obtained on machine-drawn milk were due to contamination from the machine itself, and this was proved by the reduction in counts when the machines were given adequate treatment. For example, Edwards [51] reduced the counts on machine-drawn milk from 203,000 to 1,208,000 per ml to from 1,407 to 1,776 by special treatment of the machine. More recently, Jensen and Bortree [55] lowered the counts from 47,000 to 620,000 to from 900 to 7,200 by boiling the inflations in 0.5% lye solution.

That very low-count milk can be produced with machine milking has been shown repeatedly. Milk of the finest possible bacteriological quality often is machine drawn. All that is needed is adequate care of the machines.

Sources of the Bacteria in Machine-drawn Milk. The more or less complicated construction of milking machines makes cleaning difficult, unless special methods are used, and carelessness is quickly reflected in the bacterial content of the milk.

Ruehle *et al.*[56] found that the chief sources of bacteria added to milk by a machine were the teat cups and rubber tubes although pails were important in some cases. Air contamination was unimportant. Dropping the teat cups on the floor caused relatively large amounts of dirt in the pails, but analyses of sterile water drawn through the machines did not show the high bacterial counts that might be expected since the values were not above 25,000 per ml. Macy [57] listed the chief sources of contamination of machine-drawn milk, in order of importance, as follows: (1) Teat cups, (2) rubber tubes, (3) pails and heads, (4) condensation water from vacuum line, (5) stable air, and (6) filth on teat cups if dropped on the floor. Flake [58] examined over 1,000 milking machines on farms and noted that the parts most often found dirty, listed in order of frequency, were: (1) Pail head, (2) inflations or teat cups, (3) pail, (4) long milk tubes, and (5) claw. In many instances the gasket on the pail head was not removed and cleaned.

Extent of contamination due to any particular part of a milking machine is rather difficult to determine because of the construction and also because of failure to remove all bacteria from a part. Studies on individual parts have been reported [59, 60, 61] but the methods for assessing contamination have varied.

Dahlberg *et al.*[60] determined bacterial contamination of a milking machine by drawing 5 liters of sterile water through it, using a sterile "tin udder." Individual counts of 10,000 or average counts of 5,000

per ml of water were considered satisfactory. In order to obtain some idea of the number of bacteria in a teat cup assembly, 5 lots of sterile water of 5 liters each were "milked" through a machine. The bacterial counts per milliliter of the lots were 32,000, 9,000, 5,000, 4,000, and 5,000, respectively, indicating that bacteria are removed with difficulty from such an assembly. Ellingsen [61] obtained counts of over 100,000 per ml on rinses from 17.39% of 69 milking machine pails and 16.87% of 83 teat cups examined. Churchill and Mallmann [62] rinsed dirty air-line hoses with 20 ml sterile milk and obtained counts from 910,000 to 5,780,000 per ml of rinse. Substitution of a sterile air-line hose for a dirty one, however, gave no apparent change in the bacterial counts on milk.

Importance of condensation water from the vacuum line as a source of bacteria is evident from early studies.[63,64] Investigations by Breed and Bright [65] indicated that condensation in the vacuum line did not result in extensive contamination but was of significance in producing high-grade milk. Parkin [66] listed the sources of vacuum line contamination as: Raising dust during operation of the vacuum pump, sucking bedding into the machine, unclean udders, leaky inflations, filling milking machine pails too full or tipping them, improperly placed relief valves, and sucking milky rinse water into the line.

Dirty vacuum lines frequently contain material in which organisms develop rapidly, especially during the warm months; this is evident from the odor. Dirty material returning from the vacuum line may interfere with proper closing of check valves.

Bacterial Flora of Machines. Various types of organisms are present in milking machines; presumably the method of cleaning or sterilizing influences the species in a machine. Hastings and Hoffmann [50] compared the types of bacteria in machine- and hand-drawn milk and found no marked difference. Robertson [67] isolated bacteria from various parts of machines and noted that gram-negative rods and *S. lactis* regularly were present; this was particularly true of machines in unsatisfactory bacteriological condition. White, gram-positive cocci were common in machines treated with a brine hypochlorite solution, and alkali-forming rods were predominant in tubes held in old sterilizing solutions of inadequate strength. Spore formers rarely were isolated and only a few *Escherichia-Aerobacter* cultures were obtained. Molds (primarily *G. candidum*) and yeasts were found in accumulations of old milk in various parts of machines. Lochhead and Johns [68] noted no significant differences in the bacterial flora of low-count milk drawn by machine and by hand. Inadequate washing

of the milk tube system resulted in an increase in alkaline and proteolytic types of bacteria and a decrease in inert types. Hoy and Rowlands [69] found that the flora of old milk tubes consisted almost entirely of micrococci.

Pseudomonas species have been found on rubber milking machine parts washed with nonionic detergent quaternary solution followed by dry storage.[60] Claydon [70] made a field study of the types of bacteria associated with teat cup inflations stored in lye solutions. *S. lactis* types comprised a large proportion of the flora of rubber parts, considerably more so than with other dairy utensils examined at the same farm. Laboratory experiments with teat cup inflations artificially contaminated with raw milk and later stored in 0.25% lye solution for periods up to 20 hr indicated that *S. lactis* was the predominant type surviving. In some instances *S. lactis* could not be demonstrated before the lye treatment. Similar experiments with a 0.5% lye solution resulted in survival of only *Bacillus* species after 1 hr. The data emphasize the importance of using a lye solution of proper strength.

Care of Milking Machines. Various methods of adequately treating milking machines have been developed and the primary problem is to have one of them followed in detail. The care of machines involves three distinct procedures, (1) rinsing, (2) washing, and (3) sterilizing.

A machine unit should be rinsed as soon as the milking is completed. With the vacuum pump on, at least 2 gal of cold or lukewarm water is drawn through each unit. The teat cups are lowered and raised in and out of the water to obtain an air-brush effect. After rinsing, the unit may be either (1) flush-washed with hot water containing a detergent, using the same procedure as in rinsing, or (2) it may be disassembled and all parts brush-washed. If the unit is flush-washed, the outer surfaces of the milk tube system, pail head, pail, and claw should be brush-washed, rinsed with hot water, and placed on a drying rack; then the rubber parts are treated with a chemical solution for cleaning and sterilizing. If the rubber parts are brush-washed, they may be sterilized with either a chemical solution or by heat; after heat treatment the rubber parts commonly are dry stored on a rack.

Chemical Treatment. With chemical treatment, the teat cups and tubes either are (1) suspended in a rack equipped with a container of sterilizing solution that permits filling the cups and tubes, or (2) the rubber parts are immersed in a container of solution. With the rack method, the solution is allowed to flow slowly into the cups and

tubes and fill them completely; with the immersion method, an earthenware or glazed porcelain container is filled with sterilizing solution and the inflations and tubes lowered slowly into it so that air is driven out and the solution is in contact with all surfaces. When needed, the inflations and tubes are drained and rinsed with water to remove the sterilizing solution. With the rack method, the solution is drained

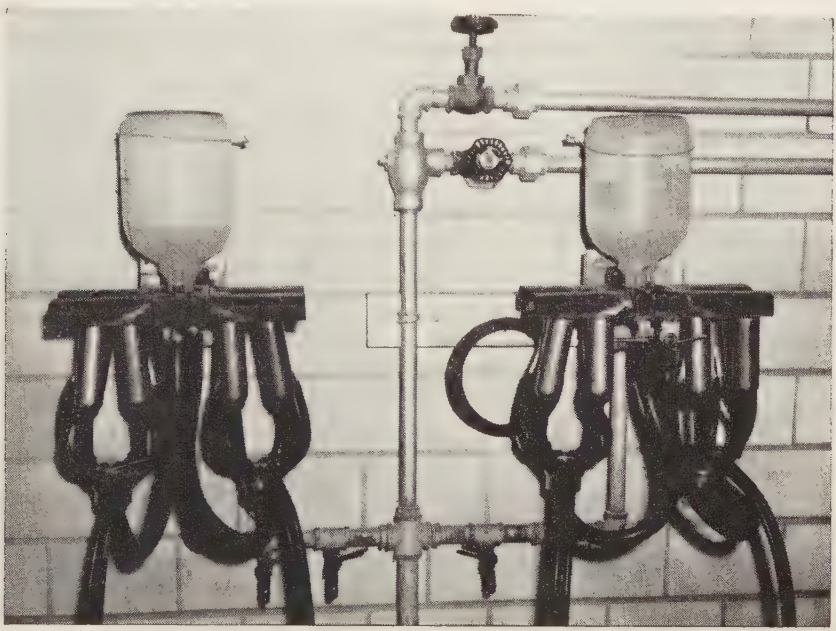


Figure 20. Solution racks for teat cup assemblies.

from the teat cups and tubes and discarded; with the immersion method the solution is discarded at frequent intervals and kept up to proper strength at all times.

Effectiveness of a 0.3 to 0.5% lye (sodium hydroxide) solution for sterilizing, as recommended by Parfitt,[71] has been thoroughly established. The chemical is cheap, readily available, relatively stable, and prevents formation of a precipitate on the rubber parts. Johns[72] reported that flush washing of the milk tube system plus storage in 0.5% lye solution gave satisfactory results over a 12-yr period. Jensen and Bortree[73] checked the action of various sanitizing solutions on synthetic rubber inflations by weighing them when new and after treatment. Of the various chemicals used, there was appreciable gain

in weight on storage in all solutions except lye. The greatest gain in weight occurred with the more highly active chlorine solutions.

Solutions of chlorine compounds, especially hypochlorites, have been used widely with milking machine parts; usually from 100 to 200 ppm available chlorine are recommended. Fay *et al.*[74] reported that machine tubes and teat cups can be sterilized by chlorine solutions testing 100 ppm available chlorine or 0.3% lye; with immersion, the chlorine solutions were less satisfactory than lye. Dahlberg *et al.*[60] obtained high rinse counts when rubber parts were stored in a chlorine solution (300 ppm) and the parts were not as clean as with lye. Other investigations[75] indicate that the flush method of cleaning does not give the same results if either chloramine-T or hypochlorite solution is used in place of lye; semisolid material accumulates in the rubber parts when chlorine solutions are used. Chlorine solutions also are reported to soften and cause peeling of rubber.[73]

Other chemicals have been suggested for the treatment of machine tubes and teat cups. Rogers and Evans[76] employed trisodium phosphate and a small amount of sodium chromate in water; poor results were obtained part of the time due to gradual build-up of spores in the solution. Johns[75] replaced lye with trisodium phosphate or sodium metasilicate for use in a solution rack with good results. Mallmann *et al.*[77] reported that a detergent mixture containing sodium metaphosphate prevented formation of milkstone on both metal and rubber parts of machines. Wetting agents also are being suggested for use in washing milking machines; after washing, hot water (160 F) is drawn through the teat cup assembly and the parts are drained and allowed to dry.[78]

An apparatus for cleaning the teat cup assembly of milking machines by circulation was used by Møller-Madsen.[79] It consists of a manifold which is placed over the teat cups so that solutions can be circulated back and forth through the assembly. Manual washing of an assembly, which consisted of brushing with a wetting agent and sterilizing with chlorine, gave bacterial counts on the milk over 4 days from 30,000 to 128,000 per ml. When mechanical cleaning was introduced, using acid and alkaline detergents alternately, the counts decreased rapidly and then over 7 days ranged from 2,310 to 4,800.

Heat Treatment. Immersing the teat cups and tubes in water and raising the temperature has been used with milking machines and found effective.[80] Heating a machine unit in water and then holding it under various conditions[81] or using steam sterilization[82] also have been employed successfully. With heat treatments generally there

is an effect on the rubber. This may not be serious, especially with certain types of rubber, but heat may cause rubber to stick to metal parts unless the units are taken apart frequently. Storage of inflations in 0.5% lye solution has given results comparable to steam sterilization.[83]

Henderson *et al.*[84] found that the heat-treatment of milking machine rubber parts, using water at 185 F and leaving the parts in the water for 20 min, was the most satisfactory method of heat sterilization used. When the parts were heated to 170 F and held for 20 min, or when they were left in the water to cool gradually until the next milking, there was evidence of growth of thermophilic bacteria. Hypochlorite solution containing 200 ppm chlorine, or 0.3 or 0.5% lye solution, gave bacterial counts somewhat higher than treatment at 185 F but was effective in controlling thermophilic organisms. With different methods of heat treatment, life of the teat cup liners was approximately the same but was 33% less than with chlorine or lye.

Because of the effectiveness and convenience of sterilizing teat cups and tubes with chemical solutions, heat treatment is not commonly used although it may be required in some areas.[85]

Holding in Cold Water. Holding the washed teat cups and tubes in a container into which cold water runs continuously has been tried as a means of keeping milking machines in good condition, bacteriologically.[56, 57, 82, 86] The method generally has not been satisfactory because of the need for low water temperatures and the tendency of water bacteria to grow on the rubber.

Precautions in Laboratory Studies. A method which is satisfactory for cleaning or sterilizing milking machines in the laboratory should be verified extensively on farms. When used on a farm, a poor method of cleaning may show satisfactory bacteriological results for some days if the equipment was in good condition at the start of the trial. Also, a satisfactory method of cleaning may fail to give excellent results at the start of a trial because the equipment was in poor bacteriological condition.[60]

According to Claydon [87] rinse and swab methods for measuring bacterial contamination of teat-cup liners may give different results than a method which employs flexing of the rubber. He described a method for determining bacterial contamination of individual liners in which a partial vacuum and pulsating action are used to surge water up and down in the liners; it gave much higher bacterial counts than a rinse-shake method. Studies [88] on a laboratory procedure for contaminating teat-cup liners indicated that bacterial counts on liners

having microscopic deterioration are markedly higher than on new liners contaminated under similar conditions. Although new liners could be rather uniformly contaminated, results obtained with them may not be applicable to used liners. For example, simplified cleaning methods might prove effective with new rubber while more thorough procedures might be necessary for used liners with inconspicuous surface breakdown.

Treatment of Milking Machines in Poor Bacteriological Condition. Frequently, a machine becomes contaminated with large numbers of bacteria, deposits of milk solids, hard water salts, etc., due to improper washing. Prior to establishing a satisfactory procedure, the deposits should be removed. Milkstone can be removed from milking machine parts by immersing in a solution of 1 part organic acid cleaner and 4 parts warm water for 20 min, followed by vigorous brushing.[89] Rubber parts of machines often become impregnated with fat which must be removed periodically. Jensen and Bortree [73] noted that the upper portion of an inflation absorbed more fat than the lower portion; presumably, this was due to a film of milk gathering between the teat and rubber which resulted in fat being worked into the rubber. Boiling the rubber parts in a lye solution (2 oz per 1 gal water) resulted in satisfactory removal of the fat and also reduced the bacterial population considerably. For example, 2 milker units from a farm having high-count milk were rinsed with 500 ml of sterile water and the water plated. One unit contained 10,500,000 bacteria, of which 7,500,000 were thermoduric, and the other contained 4,500,000 bacteria, of which 3,900,000 were thermoduric. When the units were cleaned and the rubber parts boiled in lye solution, a count of 1,000 bacteria, of which 500 were thermoduric, was obtained on each unit.

The effect of deterioration of the inside surfaces of milk tubes as a potential hazard in milking machine sanitation was emphasized by Claydon.[90] Even with used tubes having only microscopic breakdown, contamination was much greater than with new tubes. Average contamination in the used tubes was 15 times as great as in new tubes under laboratory conditions and 12 times as great under practical operating conditions using dry storage. With lye storage, the bacterial counts were lower and the difference between old and new tubes was smaller.

Dismantling the teat cup assembly and boiling the rubber parts in lye solution is necessary, but there is a difference of opinion as to how often it should be done. Undoubtedly, the frequency depends on the

daily cleaning and sterilization procedure. With satisfactory procedures, dismantling every 2 weeks is sufficient.

Care of Vacuum Lines. Parkin [66] recommended that vacuum lines be cleaned at least 3 or 4 times a year, or whenever milk enters a line. The suggested procedure for cleaning is to suck into the stallcock at the end of the line, using the stantion hose, about 2 gal of hot water containing a small amount of wetting agent. Then a can of lye is mixed with 2 gal of water and the mixture sucked into the stallcock nearest the pump, the solution being collected in the sanitary trap. The lye solution then is sucked into the next stallcock. The procedure is continued until the lye solution has been run through all stallcocks. The caustic solution is rinsed from the line by running clean hot water through all stallcocks.

Cleaning Udders with Machine Milking. Before a machine is attached to a cow, the udder commonly is brushed, wiped, or washed to reduce contamination from it. Byers and Ewalt [91] used 7 cows to determine the comparative values of water and chlorine solution (150 ppm) for washing udders and rinsing milking machine teat cups. With 490 samples each, the average bacterial count on the milk with water was 2,171 per ml and with chlorine it was 1,428.

Kesler *et al.*[92] compared the effects of various udder washes on the plate count of milk, using 2 concentrations of chlorine (200 and 400 ppm), 2 of a quaternary ammonium compound (200 and 400 ppm), and clean water. Milking was done with an autoclaved milking machine. No difference in counts could be attributed to the treatments. Also, with 25 pairs of observations, there was no significant difference in counts between night and morning milk.

Damp paper towels, damp cloths, and dry bare hands were compared by Beck and Claydon [93] for cleaning udders prior to machine milking. Differences in the plate counts and coliform counts of the milk obtained with the treatments were not statistically significant. Sediment ratings of the milk obtained averaged 0.19 mg for paper towels, 0.15 for cloths, and 0.43 for bare hands; differences between the bare hand method and either other method were highly significant.

Pipe Line Milkers. Pipe line milkers, in which a pipe line is used to convey the milk from the milking machines to the milk house, are proving very satisfactory. The type of installation largely determines the method of cleaning.

With certain installations, the line and teat cup assembly can be cleaned and sterilized together by the circulation of solutions from a

tank. A manifold is used to hold the assembly and is connected to the pipe line. The entire system first should be flushed with water (100 F) until the return water is clear. Then an alkaline detergent solution, maintained at 150 to 160 F, is circulated for about 30 min. All stallcocks should be brush-washed. Finally, the system is rinsed with clean water at 125 to 135 F to remove the detergent solution. Before use, a chlorine solution (200 ppm) is circulated for at least 20 min. An organic acid detergent may be substituted for the alkaline detergent about once each week to prevent mineral deposits. Releasers, milk pumps, receiving vessels, inflations, etc., should be dismantled once each week, or oftener if necessary, and hand brushed. Such a cleaning procedure has resulted in low bacterial counts with a number of installations.

Quality of the Milk. The bacteriological quality of milk obtained with a pipe line milker depends largely on the care used in cleaning and sterilizing. Bacterial counts on milk from a farm using a pipe line milker and bulk storage tank were reported by Moseley.[94] The pipe line was cleaned and sanitized after each milking but had not been dismantled for several months. During the warm months the raw milk had plate counts of 650 to 17,000 per ml; 91% of the samples contained $<3{,}000$. Laboratory pasteurized counts on these samples ranged from 10 to 800 per ml; 78% of the samples contained <100. Another farm, with similar facilities but where there was carelessness in cleaning and sterilizing the equipment, had total counts of 80,000 to 360,000 per ml, with the majority of counts $>150{,}000$.

Alexander *et al.*[95] studied the quality of milk produced under four different systems of milking. Milk of acceptable quality was produced with either an open (milk exposed to air of milk room while dropping into open strainer) or closed (closed system from cow to can) combine system, a conventional milking machine and manual carry-away system, or a permanent cow-to-cooler pipe line system. Average counts for the evening and morning milkings were respectively as follows: Open combine 11,000 and 14,000 per ml; closed combine 7,100 and 5,600; conventional milking machine 4,850 and 5,100; and pipe line cleaned in place 8,750 and 8,075. Later experiments[96] indicated no significant difference in the bacteriological quality of milk handled by permanent pipe lines constructed of stainless steel or Pyrex glass. Milk pipe lines were sanitized effectively by either a chlorine solution (200 ppm) or hot water (185 F). When a milk pipe line was allowed to become contaminated excessively by use of

improper procedures, it was not necessary to dismantle the pipe line to restore it to satisfactory bacteriological condition.

The quality of the milk on two farms using stainless steel pipe lines to carry milk from the barn to bulk cooling tanks was determined by Hunter *et al.*[97] The pipe line on one farm was designed for dis-

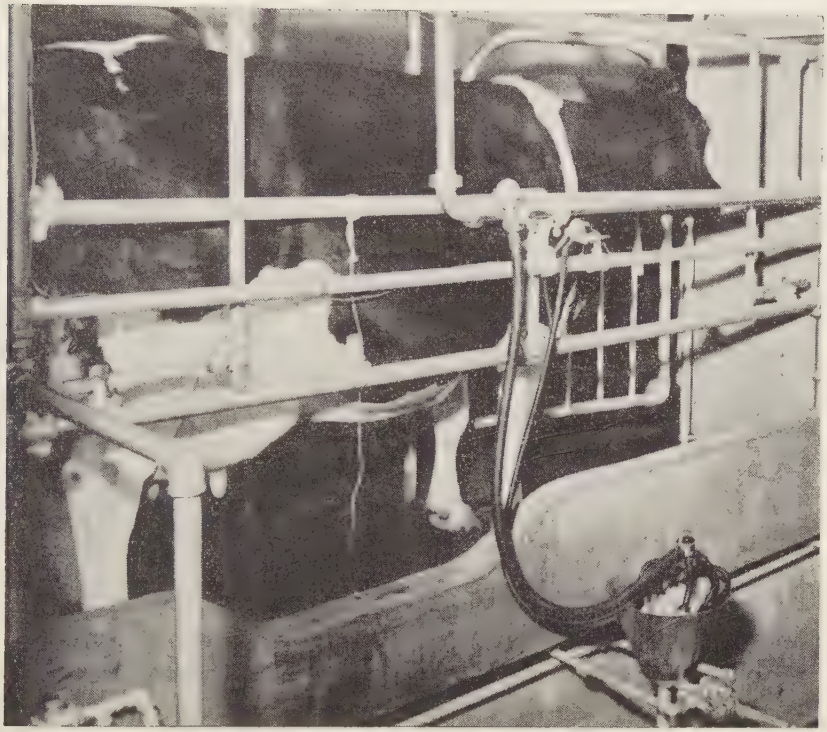


Figure 21. Portion of a milking parlor.

mantled cleaning and that on the other farm for in-place cleaning. The bacteriological quality of milk produced on both farms was excellent. The pipe line designed for dismantled cleaning was taken down daily, twice weekly, weekly, and biweekly for a time and when not dismantled was cleaned in place. The bacterial population on the surface of the pipe line increased as the time between dismantling was increased, but this increase was not reflected in the bacterial count of milk passing through the line.

Hydrolytic Rancidity. Milk produced with pipe line milkers apparently develops hydrolytic rancidity more often than that produced with other milking systems. Kelley and Dunkley[98] studied, under

controlled conditions, the factors influencing lipase activation in pipe line milkers. Among the more troublesome were (1) admission of air to the milk line, (2) low rate of milk flow, (3) elevation of warm milk under vacuum with air bubbling through it, (4) inclusion of a filter and numerous fittings in the vacuum section of the line, and (5) continuous operation of a starved centrifugal pump. Variation in the susceptibility of the milk to induced rancidity also appeared to be important.

Milking Parlors. A milking room, holding only a few cows and in which cows are milked but not stabled, offers certain advantages over a conventional barn for production of high quality milk. The area is small and easy to clean. Usually, the room has impervious floors and the walls and ceiling are sealed tightly. It normally is close to the milk room, where the milk is stored and the utensils washed, so that hot and cold water are available for rinsing floors, washing udders, etc. In rooms with an elevated platform for the animals, both the operator's area and cow platforms should have trapped drains and provision for liquid waste disposal. With such facilities, scrubbing with water under pressure is easy and effective. Supplementary heat in the parlor during cold weather gives comfort to the operator and hastens drying of the floors and walls.

GENERAL CONSIDERATIONS

After milk containing relatively few bacteria has been obtained, with either hand or machine milking, the immediate problem is to limit the growth of bacteria in it. This involves essentially rapid cooling to temperatures which prevent, or greatly limit, growth of organisms that inevitably gain entrance (see Chapter 6) and is a most important factor in determining the quality of the milk coming from a farm.

BULK HANDLING OF MILK

A system which avoids the use of cans by storing the milk on a farm in a cold wall tank, where it is cooled rapidly, and then pumping it into a tank truck for transportation to the plant is being extended rapidly in many dairy areas. It [99 104] reduces and simplifies the work on the farm, commonly insures adequate cooling of the milk, and facilitates better housekeeping in the milking operation. Transportation costs are reduced and, because the milk is well cooled, less than daily collection can be adopted easily. At the plant, the system sim-

plifies and reduces the cost of receiving the milk and there is less milk loss. Since a change to the bulk system on a farm requires considerable investment, it is not practical for a small milk producer.

In general, bulk milk handling results in lower bacterial counts than when cans are used. Woodruff [105] reported that the can milk received at a plant on 2 successive days had plate counts of 200,000 and 160,000

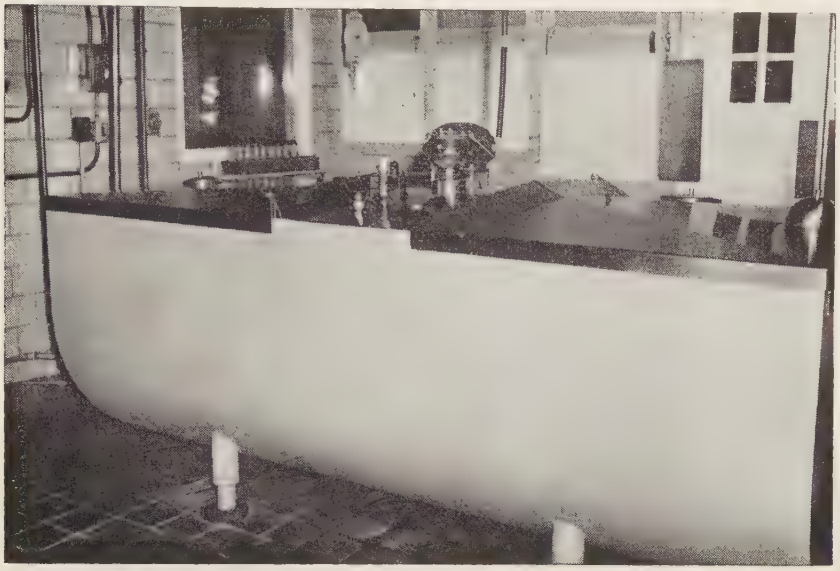


Figure 22. Farm bulk milk tank.

per ml, whereas on the same days the counts on the bulk milk were <30,000; temperatures of the can milk were 53 and 45 F and those of the bulk milk were 39 and 38 F. When 2 routes were converted from cans to bulk systems,[101] there were reductions in count of 65 and 55%. Marth *et al.*[106] found that milk produced and handled with the bulk system studied had a low plate count, usually 3,000 to 5,000 per ml and rarely over 10,000. The milk could be held in the tanks for 2 days with no significant increase in the count. Also, there was no significant increase in the count during transportation of the milk in a tank trailer. It was found that the period of agitation required to permit representative sampling of milk in tanks for bacteriological tests varied with the type of tank, quantity of milk, period the milk had been undisturbed, and location from which the sample was taken. Even when the milk in a tank was least homogeneous, 60 sec of agi-

tation gave a representative sample from one tank and 90 sec from the other.

Johnson *et al.*[107] made 51 comparisons of the bulk and can systems of handling milk over a 15-month period, using the bulk system one day and the can system the next. Plate counts with the bulk system ranged from 5,000 to 200,000 per ml (log average 17,400) while those with the can system varied from 7,600 to 390,000 (log average 54,700). With the bulk system, only 6 of the 51 counts were >50,000 per ml; with the can system, 25 counts were >50,000 and 5 were >200,000. Coliform counts on milk handled with the 2 systems showed little difference although those on the bulk milk were slightly lower.

Without adequate cleaning and sanitizing of the equipment, high counts are obtained on milk handled in bulk, just as when cans are used. This is evident from the following data obtained by Moseley[94] on milk from two producers, each using a pipe line milker and bulk cooling tank.

Comparative Counts on Milk from Two Producers, Each Using a Pipe Line Milker and Bulk Cooling Tank

Sample No.	Plate Count per Milliliter	
	Producer 1	Producer 2
1	830	82,000
2	760	150,000
3	1,500	190,000
4	1,100	360,000
5	800	80,000

Marth *et al.*[106] used the following procedure for cleaning and sanitizing a bulk tank: (1) The tank surface was rinsed with cold water; (2) it was brushed clean with use of an alkaline detergent (every fourth day an organic acid was used instead); (3) the valves and agitators were disassembled and brushed; (4) all parts were given a final water rinse; and (5) just before milking all milk contact surfaces were sanitized with a 200 ppm chlorine spray. Swab contact tests indicated that flat open surfaces of the tank assemblies were maintained in a satisfactory condition but the valve surfaces required special care in cleaning and sanitizing.

Experience indicates that valves and connecting pipes are neglected most often in the cleaning of bulk tanks. If such parts are to be cleaned properly, suitable brushes, wrenches, etc., are necessary.

The bulk system also presents some problems.[108] It will be necessary for regulatory officials to precede the tank truck, or ride on the truck, if samples from individual farms are to be collected since commonly milk from more than one farm will be in the tank when it

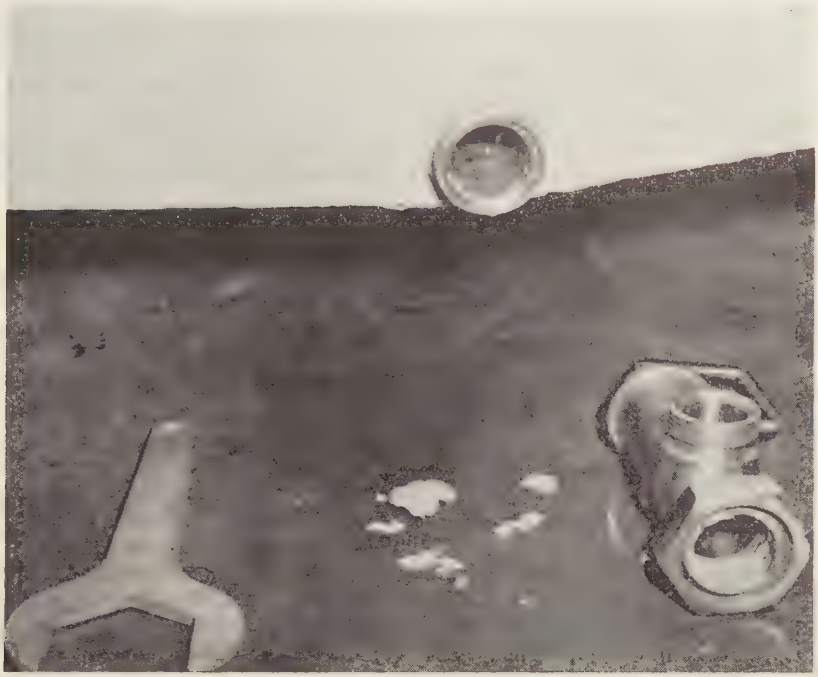


Figure 23. Outlet valve, on farm bulk milk tank, that was not properly cleaned.

reaches the plant. The truck driver will need to be experienced in judging milk for flavor so that milk with poor flavor will not be mixed with milk having satisfactory flavor, to the detriment of the latter. Also, the driver must avoid carelessness in handling hose, pipe connections, etc.

A very important question in connection with bulk tanks on farms is their calibration to the satisfaction of both the seller and purchaser of the milk. This and similar installation problems are now being given much attention by milk sanitarians.[109]

REFERENCES

1. *Proc. Soc. Promotion Agr. Sci.* 110 (1899).
2. *J. Appl. Mic.*, **1**, 205 (1898).
3. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 178 (1900).
4. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 27 (1913).
5. *S. Dak. Agr. Expt. Sta. Bull.* 218 (1926).
6. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 132 (1928).
7. *J. Dairy Sci.*, **16**, 79 (1933).
8. *Proc. Utah Acad. Sci.*, **18**, 9 (1941).
9. *Indian J. Dairy Sci.*, **2**, 89 (1949).
10. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 165 (1930).
11. *Bimonthly Bull. N. Dak. Agr. Expt. Sta.*, **13**, 21 (1950).
12. *Intern. Assoc. Dairy and Milk Inspectors, 24th Ann. Rept.* 178 (1935).
13. *Wis. Agr. Expt. Sta. Research Bull.* 6 (1909).
14. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **13**, 281 (1904).
15. *J. Dairy Sci.*, **13**, 499 (1930).
16. *Can. Dept. Agr. Publ.* 524 (1939).
17. *W. Va. Agr. Expt. Sta. Bull.* 134 (1911).
18. *J. Dairy Sci.*, **15**, 163 (1932).
19. *Wis. Agr. Expt. Sta., 21st Ann. Rept.* 164 (1904).
20. *J. Infectious Diseases*, **18**, 437 (1916).
21. *Compt. rend.*, **234**, 255 (1952).
22. *Creamery Milk Plant Monthly*, **3**, 11 (Feb. 1915).
23. *J. Bacteriol.*, **2**, 185 (1917).
24. *J. Dairy Research*, **7**, 63 (1936).
25. *Conn. (Storrs) Agr. Expt. Sta., 18th Ann. Rept.* 66 (1906).
26. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 409 (1915).
27. *Ill. Agr. Expt. Sta. Bull.* 236 (1921).
28. *Abstr. Bacteriol.*, **2**, 6 (1918).
29. *J. Dairy Sci.*, **6**, 479 (1923).
30. *J. Dairy Sci.*, **1**, 303 (1917).
31. *J. Dairy Sci.*, **29**, 5 (1946).
32. *J. Assoc. Offic. Agr. Chemists*, **36**, 301 (1953).
33. *U. S. Dept. Agr. Bull.* 642 (1918).
34. *J. Dairy Sci.*, **31**, 325 (1948).
35. *Standard Methods for the Examination of Dairy Products* (Last ed.). American Public Health Association, Inc., New York, N. Y.
36. *J. Milk Technol.*, **8**, 134 (1945).
37. *Ia. Agr. Expt. Sta. Bull.* 300 (1933).
38. *Ill. Agr. Expt. Sta. Bull.* 204 (1918).
39. *J. Dairy Sci.*, **2**, 487 (1919).
40. *J. Dairy Sci.*, **3**, 540 (1920).
41. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 365 (1913).
42. *Ill. Agr. Expt. Sta. Bull.* 230 (1920).
43. *J. Dairy Sci.*, **4**, 79 (1921).
44. *Food Research*, **11**, 327 (1946).
45. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 51 (1908).
46. *Am. J. Public Health*, **4**, 185 (1914).
47. North, C. E., *Farmers' Clean Milk Book*, 113 (1918). John Wiley and Sons, Inc., New York, N. Y.
48. *U. S. Dept. Agr. Research Service Mktg. Rept.* 64 (1954).
49. *Ontario Agr. Coll., 23rd Ann. Rept.* 128 (1897).
50. *Wis. Agr. Expt. Sta., 24th Ann. Rept.* 214 (1907).
51. *Ontario Agr. Coll. Bull.* 159 (1907).
52. *Pa. Agr. Expt. Sta. Ann. Rept. 1907–1908*, 146 (1908).
53. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 317 (1909).
54. *S. Dak. Agr. Expt. Sta. Bull.* 144 (1913).
55. *J. Dairy Sci.*, **29**, 849 (1946).

56. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 450 (1918).
57. *Univ. Minn. Agr. Ext. Div. Sp. Bull.* 138 (1931).
58. *Butter Cheese Milk Products J.*, **41** (12), 26 (1950).
59. *J. Dairy Sci.*, **31**, 331 (1948).
60. *J. Milk and Food Technol.*, **13**, 5 (1950).
61. *Norsk Landbruk.*, **16**, 258 (1950).
62. *J. Milk and Food Technol.*, **13**, 137 (1950).
63. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 47 (Part 1) (1907).
64. *Proc. Certified Milk Prod. Assoc. Am., 7th Ann. Conv.*, 28 (1914).
65. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 488 (1921).
66. *Milk Plant Monthly*, **38** (12), 68 (1949).
67. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 105 (1924).
68. *Can. Dept. Agr. Bull.* 127 (1929).
69. *Proc. Soc. Appl. Bacteriol.*, 40 (1948).
70. *J. Dairy Sci.*, **34**, 480 (1951).
71. *Purdue Agr. Expt. Sta. Bull.* 348 (1931).
72. *J. Milk Technol.*, **6**, 274 (1943).
73. *J. Dairy Sci.*, **29**, 849 (1946).
74. *J. Dairy Sci.*, **18**, 239 (1935).
75. *Sci. Agr.*, **13**, 460 (1933).
76. *J. Dairy Sci.*, **19**, 733 (1936).
77. *J. Dairy Sci.*, **23**, 621 (1940).
78. *J. Milk and Food Technol.*, **11**, 195 (1948).
79. *Proc. Intern. Dairy Congr., 13th Congr.*, **2**, 189 (1953).
80. *J. Dairy Sci.*, **3**, 33 (1920).
81. *J. Agr. Research*, **34**, 27 (1927).
82. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 144 (1927).
83. *Proc. Soc. Appl. Bacteriol.*, 40 (1948).
84. *J. Dairy Sci.*, **17**, 475 (1934).
85. *Proc. Intern. Dairy Congr., 12th Congr.*, **1**, 215 (1949).
86. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 492 (1922).
87. *J. Dairy Sci.*, **36**, 391 (1953).
88. *J. Dairy Sci.*, **36**, 707 (1953).
89. *Milk Plant Monthly*, **39** (2), 68 (1950).
90. *J. Milk and Food Technol.*, **17**, 359 (1954).
91. *J. Dairy Sci.*, **26**, 277 (1943).
92. *J. Dairy Sci.*, **31**, 179 (1948).
93. *J. Dairy Sci.*, **34**, 593 (1951).
94. *Milk Ind. Foundation Conv. Proc.*, **5**, 11 (1953).
95. *J. Dairy Sci.*, **35**, 874 (1952).
96. *J. Dairy Sci.*, **36**, 303 (1953).
97. *J. Milk and Food Technol.*, **17**, 43 (1954).
98. *J. Milk and Food Technol.*, **17**, 306 (1954).
99. *J. Milk and Food Technol.*, **15**, 294 (1952)
100. *Milk Plant Monthly*, **42** (3), 23 (1953).
101. *Milk Plant Monthly*, **42** (3), 18 (1953).
102. *J. Milk and Food Technol.*, **16**, 260 (1953).
103. *J. Milk and Food Technol.*, **17**, 210 (1954).
104. *J. Milk and Food Technol.*, **17**, 223 (1954).
105. *Am. Milk Rev.*, **13** (7), 6 (1951).
106. *J. Milk and Food Technol.*, **17**, 86 (1954).
107. *Okla. Agr. Expt. Sta. Bull.* B-436 (1954).
108. *Am. J. Public Health Yearbook 1952–53 (Part 2)*, **43**, 107 (1953).
109. *J. Milk and Food Technol.*, **17**, 58 (1954).

Washing, Bactericidal, and Preserving Reagents

5

Various chemical compounds are employed in the dairy industry. Some are used in washing dairy equipment, others in the sterilization (practical) of equipment, and still others in the preservation of milk, cream, etc.

WASHING COMPOUNDS

Compounds used in washing dairy equipment generally are mixtures of several chemicals. Somers [1] noted that the effectiveness of a washing compound is determined by the degree to which it fulfills the following requirements: (1) High wetting or penetrating action, to effect rapid removal of soil; (2) good deflocculating or dispersing action, to bring deposits into suspension; (3) high emulsifying power for oils; (4) good rinsability, to permit removal of soil and detergent; (5) water conditioning or sequestering properties in alkaline solution, to prevent calcium and magnesium salts of the water from depositing on equipment; (6) dissolving and neutralizing power, to remove tenacious deposits and saponify fats; and (7) low corrosive action on the surfaces for which they are used. Also, in certain applications it is desirable that the compounds have high active alkalinity and bactericidal properties.

Compounds Used in Formulations. The general groups of chemicals used in the formulation of washing compounds are the alkalies, complex phosphates, wetting agents, acids, and chelating agents.

Alkalies. Sodium hydroxide (lye or caustic soda) gives a *p*H of about 13.3 in a 10% solution [1] and has the highest alkalinity of the alkalies. It readily saponifies fat and has rapid dissolving power, but it is irritating to the skin and corrosive to most metals other than stainless steel. Sodium hydroxide serves as a detergent and bactericide for bottles, rubber parts of milking machines, rubber parts of bottle fillers, etc. Sodium carbonate (soda ash) is similar to sodium hydroxide in detergent properties but has lower alkalinity (*p*H 11.5 in a 10% solution) and therefore less dissolving power. The compound is useful as a buffer in alkaline detergent solutions. Sodium metasilicate gives a *p*H of about 12.4 in a 10% solution and is used extensively as a corrosion inhibitor for sodium hydroxide. It can be used alone as a washing compound because it has good wetting, rinsing, emulsifying, and deflocculating properties. Trisodium phosphate gives a *p*H of about 11.9 in a 10% solution. It is used as a general cleaner and water softener. Wetting properties of the compound are rather low but its rinsing, emulsifying, and deflocculating properties are quite high. The compound softens water by precipitation of hard water salts and such precipitation may be objectionable. Trisodium phosphate, like the other alkalies, is corrosive to aluminum and tin.

Complex Phosphates. The complex phosphates used in formulations are tetrasodium pyrophosphate, sodium tripolyphosphate, sodium tetraphosphate, sodium decaphosphate, and sodium hexametaphosphate; 10% solutions of the respective compounds give *p*H values of 10.2, 9.6, 7.6, 7.2, and 6.8.[1] The primary function of the complex phosphates is that of water conditioning which is accomplished by the formation of soluble compounds of calcium and magnesium. The compounds have excellent emulsification, dispersion, and suspension properties. The water conditioning properties of the phosphates vary; sodium tripolyphosphate sequesters magnesium more effectively than calcium, and sodium hexametaphosphate sequesters calcium more effectively. The complex phosphates are unstable in hot solutions and in the presence of strong alkalies. Tripolyphosphate is the most stable to heat and hexametaphosphate the least stable.

Wetting Agents. The alkyl aryl sulfonates are used extensively as wetting agents in formulations. Sulfated alcohols are employed

also; they are less soluble in cold water and have less defatting action on the skin than the alkyl aryl sulfonates.

Acids. Some of the acids used in formulations include phosphoric, tartaric, citric, gluconic, etc. Little [2] calculated the *p*H values of 0.5% solutions of the acids from their ionization constants as follows: Phosphoric (85%) 1.67, tartaric 2.17, citric (1 H_2O) 2.28, and gluconic (50%) 2.76. The acids are used to prevent, or remove, deposits of hard water salts and/or milk constituents. Also, they may be combined with a wetting agent and used as a washing compound.

Chelating Agents. The chelating agents are organic sequestering agents which behave similarly to the polyphosphates, but they have the advantage of being stable to heat. Ethylenediamine tetraacetic acid is an important member of this group.

Bactericidal Action of Alkalies. The washing powders used in dairy plants have a destructive action on organisms, especially when used in strong solutions and at washing temperatures. This action is a factor in the value derived from washing powders in the cleaning of equipment, and in certain bottle washers it is very important. Levine *et al.*[3] studied the bactericidal action of sodium hydroxide, sodium carbonate, and trisodium phosphate on dried bacterial spores at 156.2 to 158 F and concluded that factors other than *p*H were involved. The investigators [4] also reported results indicating that the presence of sodium carbonate enhanced the bactericidal powers of sodium hydroxide and suggested that undissociated sodium hydroxide, rather than *p*H, may be the controlling factor in destruction of organisms. It was noted [5] that addition of sodium chloride, sodium carbonate, or trisodium phosphate to sodium hydroxide markedly decreased the killing time at 122 and 140 F; as the concentration of salts added to the hydroxide was increased, killing time was decreased but at a decreasing rate.

Mudge and Lawler [6] studied the action of alkali solutions on the bacteria found in milk bottles returned to a plant. The organisms were obtained by shaking dirty bottles with sterile water, which then was collected in a container; suspensions of organisms thus obtained were exposed to various concentrations of alkali. Hydroxyl-ion concentration was an important factor in the bactericidal action, as were time and temperature. Myers [7] reported that commercial washing powders giving solutions of high *p*H were distinctly more effective as bactericides than those giving solutions of low *p*H, a powder containing chlorine being an exception. Solutions that had low *p*H values and, accordingly, weak bactericidal action became more effective

when the *p*H was increased by addition of sodium hydroxide. Bactericidal efficiency of the washing powders was not decreased to the same degree by dilution. A powder containing chlorine showed a very slight decrease in bactericidal power when diluted considerably, and the others showed varying decreases. Decreases in efficiency in these alkaline solutions appeared to be correlated closely with decreases in hydroxyl-ion concentration when considered in reference to dilutions of the same powder.

Stark *et al.*[8] found that when sodium hydroxide was used alone in the soaker tank of a bottle washer, in concentrations of 0.9 to 4.2% and at temperatures of 120 to 170 F, >55% of the bottles tested failed to show any colonies of bacteria. The average colony count per half-pint bottle never exceeded 15, even though the bottles failed to rinse well. Addition of trisodium phosphate to sodium hydroxide solutions did not improve the bacterial counts of the bottles, but addition of tetrasodium pyrophosphate gave better rinsing and less variation in bacterial counts from day to day. Foter and Finley[9] determined the bactericidal efficiency of 6 alkaline can-washing compounds. Solutions containing 0.05 to 0.1% alkali (Na_2O) had little or no bactericidal effect. Alkali concentrations of 0.15 to 0.2% markedly increased the bactericidal effect with two of the compounds.

Bactericidal Action of Wetting Agents. Various wetting agents have an inhibiting effect on organisms which may vary from one organism to another. For example, Cowles[10] concluded that sodium alkyl sulfates exert a pronounced inhibitory effect on gram-positive organisms but not on gram-negative organisms. Birkeland and Steinhaus[11] found that sodium lauryl sulfate possessed a high degree of bacteriostatic activity; growth of gram-positive bacteria and of molds was definitely inhibited, whereas most gram-negative bacteria grew freely in the presence of the compound.

Burgwald[12] noted that when wetting agents were used in combination with caustic soda in soaker-type washers there were reductions in (1) the number of cloudy bottles, (2) the amount of residual water and alkali in bottles, (3) the amount of carryover from the soaker tank to the rinse tank, and (4) the average bacterial count of the bottles. The optimum amount of wetting agent appeared to be from 0.045 to 0.06%. The average bacterial count of bottles was reduced by nearly 70% when wetting agents were used. Tenney[13] found that 0.15% alkyl aryl sulfonate was sufficient to give excellent wetting and rinsing properties to sodium hydroxide solutions. Also, the wetting agent decreased the time for destruction of organisms in the

sodium hydroxide solution; for example, a 2% sodium hydroxide solution at 95 F required 30 min to destroy *Penicillium glaucum* whereas it was destroyed in <12 min under the same conditions when 0.15% alkyl aryl sulfonate was added.

Bactericidal Action of Acid Cleaners. A preparation containing acid was studied by Frayer [14] and found to exert a bactericidal effect, within the range of concentrations used, which was independent of any detergent value as well as of the temperatures recommended by the manufacturer as a necessary part of the cleaning and sterilizing process.

Certain plants alternately use acid detergent and alkaline detergent in the can washer. If the washer is not freed of alkali, hard water salts, etc., before acid is added, the reaction of the washing solution may be neutral or slightly alkaline. Foter and Finley [9] obtained cans with an alkaline reaction when acid detergent was used. The bactericidal efficiency of the acid cleaner was greater when the cans had an acid reaction.

BACTERICIDES

Hot water and steam are used widely for the destruction of organisms in dairy utensils and equipment, and they are very effective when properly applied. Chemical bactericides also are employed and provide a method of treatment that is applicable when hot water or steam is inadvisable or not available and, in certain cases, when it is desirable to treat equipment rapidly just before use. Chemical treatment of milk bottles has proved useful in certain instances when a commercial washer or steam chamber was not available; frequently, it is employed as a final step in the washing of bottles with modern equipment. Chemical sanitizers also have been advocated for equipment that is difficult to clean and sterilize because of its complicated construction.

Chlorine Compounds. Chlorine compounds, including chlorine gas, hypochlorites, chloramine-T, and dichlorodimethylhydantoin have been used as bactericides. The materials are actively bactericidal, and the odors are not objectionable and do not influence the flavor of dairy products when used in normal amounts. In some instances a chlorine compound is combined with a washing powder to increase the destruction of organisms during the washing process.

Chlorine Gas. Chlorine gas generally is considered to be the cheapest source of chlorine. The gas can be passed into water to destroy

micro-organisms in it, or the water can be used as a rinse for utensils. The gas dissipates rather rapidly from water, and this is an advantage in some instances (chlorination of water) and a disadvantage in others (treatment of dairy equipment). Sodium or calcium hypochlorites can be prepared from chlorine gas.

Hypochlorites. The hypochlorites are used more extensively as bactericides than any of the other chlorine compounds. They are readily available, relatively cheap, and act rapidly. Sodium and calcium hypochlorites are marketed under various trade names; the preparations contain from about 3 to 70% available chlorine.

Chloramine-T. Chloramine-T (sodium paratoluenesulfonchloramide) and dichloramine-T (paratoluenesulfondichloramide) are organic compounds which release chlorine somewhat more slowly than the hypochlorites. Chloramine-T is the more widely used of the two compounds because of its greater solubility in water; dichloramine-T is soluble only to the extent of 1 part in 100,000.

Dichlorodimethylhydantoin. 1,3-dichloro-5,5-dimethylhydantoin is used as a bactericide in combination with neutral inorganic salts, a wetting agent, and an acidic compound.[15] The commercial preparation contains about 16% available chlorine and provides solutions of *p*H 5.8 to 7.0, depending on the water supply. It is sufficiently soluble in water to give solutions containing 1,000 ppm available chlorine.

Factors Influencing the Bactericidal Activity of Chlorine. A number of factors influence the bactericidal activity of chlorine compounds, such as concentration, *p*H, period of action, temperature, amount and character of organic matter present, etc., so that the evaluation of different compounds is complicated.

Concentration. The rate of bacterial destruction increases with increasing amounts of available chlorine when comparisons are made at the same *p*H and temperature and in the absence of organic matter. Rudolph and Levine[16] found that the average times required to destroy 99% of the spores in a suspension of *Bacillus metiens* with 25, 100, and 500 ppm available chlorine as hypochlorite were 121, 63.5, and 30 min, respectively. In general, increasing the concentration of available chlorine fourfold reduced the killing time by about 50%, and doubling the concentration reduced it by about 30%. With chloramine-T, doubling the concentration of available chlorine at constant temperature and *p*H reduced the killing time about 50%.[17]

Hydrogen-ion Concentration. Johns[18] noted that with hypochlorites the influence of *p*H is even greater than that of concentration of available chlorine, particularly in dilute solution. Solutions of

hypochlorite preparations containing 100 ppm available chlorine ranged in *p*H from 9.2 to 11.9. Investigations [16] with *B. metiens* as the test organism, and employing calcium hypochlorite at 25 ppm and 68 F, showed that the average killing times at *p*H 6, 7, 8, 9, 10, and 12.86 were 2.52, 3.6, 5.0, 18.5, 121, and 465 min, respectively. Weber [19] found that the *p*H of 3 chloramine-T solutions, made to contain 50 ppm available chlorine, varied from 6.4 to 10.5. The killing time with *M. pyogenes* var. *aureus* varied from 2.15 min with the solution at *p*H 6.4 to 372 min with the solution at *p*H 10.5.

Period of Action. Butterfield [20] compared the bactericidal efficiency of free chlorine and chloramine-T in water at *p*H 7 and 68 to 77 F. With free chlorine, 100% kill was obtained with 0.04 ppm residual chlorine in 1 min; with chloramine-T, 100% kill was not obtained with a residual of 1.2 ppm in 10 min but it was obtained in 20 min. To obtain 100% kill with the same amounts of residual chlorine, chloramine-T required about 100 times the contact period required by free chlorine. Weber [19] concluded that concentrations of chloramine-T of at least 250 ppm and at *p*H 7, or less, or 500 to 1,000 ppm at not above *p*H 7.5, appear to be as rapid in bactericidal action in the absence of organic matter as 50 ppm of the alkaline hypochlorites.

Temperature. At one time it was assumed that chlorine solutions rapidly decreased in strength when warmed. Prucha [21] found that chloramine-T and sodium hypochlorite lost little or no chlorine when exposed for 30 min at 80 to 160 F. Rudolph and Levine [16] reported that the loss due to warming has been overemphasized. With hypochlorite, they found that the killing time decreased with increasing temperature, a reduction of 40 to 60% being obtained for each 18 F rise in the range of 68 to 122 F.

Organic Matter. Unless dairy equipment that is to be treated with chlorine compounds is thoroughly cleaned, the action is influenced seriously. Prucha [21] noted that addition of a small amount of milk to chloramine-T or sodium hypochlorite promptly caused a reduction in the available chlorine, which became greater with an increase in the temperature or the amount of milk added. In the absence of organic matter, addition of 1% sodium hydroxide to sodium hypochlorite solution increased the stability, but, in the presence of organic matter, the available chlorine disappeared faster with the sodium hydroxide added. There was slower destruction of *E. coli* when small amounts of milk were added to solutions of various chlorine compounds than when the milk was absent. Cox and White-

head [22] reported that chloramino compounds, formed by the interaction of hypochlorite and protein or amino acids, have some bactericidal power against S. *cremoris*.

Action of Chlorine on Bacteria. Various investigators have studied the effect of chlorine compounds on organisms important in dairy products. Johns [23] tested 3 hypochlorite and 2 chloramine-T products against a mixed flora from milk cans and pure cultures of *E. coli*, *A. aerogenes*, S. *lactis*, and *B. subtilis*. The liquid hypochlorite preparations were effective against all the nonspore formers, whereas a dry hypochlorite preparation was effective against the gas-forming species, slower against the mixed flora, and especially slow against S. *lactis;* the slow action against S. *lactis* was considered to be due to high hydroxyl-ion concentration. The chloramine-T products acted too slowly to be considered suitable for bactericidal rinses. None of the 5 products had a noticeable effect on spores of *B. subtilis* during a 2-min exposure. Later, Johns [24] studied the efficiencies of 3 solutions of hypochlorite preparations in the destruction of 5 ropy and 2 bitter milk organisms. The less alkaline hypochlorites were extremely effective against all 7 test organisms; with a more alkaline product, the gram-positive species exhibited varying degrees of resistance.

Loveless [25] noted that, when the utensils on 20 Vermont farms were rinsed with hypochlorite solution twice daily immediately before milking, the average quality of the milk, as measured by plate counts and methylene blue tests, was materially improved. Milk handled in cans treated at the farm with a chlorine rinse, in addition to the treatment given at the plant, showed less contamination than portions of the same lots of milk handled in unrinsed cans. Milk produced under experimental conditions, where hypochlorite solutions were employed in treating utensils, compared favorably in quality with that produced in steamed utensils. When rinsed with a quick-acting hypochlorite preparation, artificially contaminated milk pails had relatively few bacteria on their surfaces.

Some nonspore-forming organisms may be relatively resistant to the action of certain chlorine compounds. It appears from the experiments of Wade *et al.*[26] that a calcium hypochlorite solution containing available chlorine in amounts usually advised for treating equipment and utensils in milk plants, and even up to 500 ppm, cannot be depended on to destroy human *M. tuberculosis*. Chloramine, in a strength of 93 to 95 ppm of available chlorine, for a period of 3 min was effective. Costigan [27] found that a hypochlorite solution of low alkalinity containing 50 ppm available chlorine destroyed *M. tuber-*

culosis in 2.5 min at 122 F, in 1 min at 131 F, and in 0.5 min at 140 F; with higher concentration of chlorine destruction was more rapid.

In considering the mechanism of the action of chlorine compounds on bacteria, Charlton and Levine [17] concluded that with hypochlorites the undissociated hypochlorous acid is the most significant bactericidal agent, whereas with chloramine-T this acid does not play the significant role.

Corrosive Action of Chlorine. The corrosive action of chlorine derivatives on metals requires consideration whenever these compounds are employed. Action of the different derivatives varies widely with the metals used in dairy equipment and is affected by various factors. Loveless [25] noted that as alkalinity increased the corrosion from hypochlorite was lowered and that chloramine-T showed little corrosive action. Botham and Dummett [28] found that sodium hypochlorite, either in the presence or absence of potassium permanganate, in a concentration of 150 ppm available chlorine and at 104 F is corrosive to metals such as aluminum, tinned copper, nickel silver, and stainless steel. The chlorine caused a pitting type of corrosion. Sodium silicate inhibited corrosion to a greater extent than either sodium hydroxide or carbonate. Bacon and Sotier [29] reported that a preparation containing dichlorodimethylhydantoin was less corrosive to silver, aluminum, and galvanized iron than sodium hypochlorite, but the hypochlorite was less corrosive to tin, brass, and black iron. Both products were similar in their action on stainless steel and copper.

Corrosion by chlorine compounds can be minimized if equipment is treated just prior to its use. Also, solutions of chlorine prepared for use from concentrated reagents should be prepared carefully to provide solutions of sufficient, but not excessive, chlorine concentrations. The dairy industry employs solutions of about 50 to 200 ppm available chlorine for bactericidal treatment of equipment. A solution of 50 ppm gives adequate bactericidal treatment to properly washed equipment.

Addition of Chlorine to Alkaline Cleaners. With the introduction of circulation cleaning it was observed that a light brown film occasionally forms on pipe lines, high-temperature short-time pasteurizers, etc. Addition of hypochlorites to alkaline cleaners has assisted in the removal of such films. Baker [30] noted that addition of sodium hypochlorite to egg albumin resulted in degradation of the protein. MacGregor *et al.*[31] found that the film left on equipment after circulation cleaning contained about 80% protein. It could be removed by adding

sufficient chlorine to an alkaline cleaner to give a solution containing 25 to 100 ppm available chlorine. The action was considered to be due to protein degradation which increased the protein solubility and permitted more effective removal.

Quaternary Ammonium Compounds. Quaternary ammonium compounds (quaternaries), in which various organic groups replace the four hydrogen atoms in ammonium chloride or bromide, have been developed for use in destruction of organisms in connection with preparation and handling of various foods. In general, they have high bactericidal activity, little odor or taste in normal concentrations, high stability, are noncorrosive, and have only low toxicity. In aqueous solutions they dissociate into two ions, one being the simple chloride or bromide and the other the nitrogen-carbon structure or cation. The latter is responsible for the bactericidal activity, and the compounds are called cationic bactericides. Since the compounds are wetting agents, their water solutions have low surface tensions and they contact and kill organisms not accessible to nonwetting bactericides. Also, they possess detergent properties.

Dunn [32] reported that a mixture of high molecular alkyl-dimethyl-benzyl ammonium chlorides showed high bactericidal efficiency toward a series of bacteria both in the presence and absence of organic matter. Specificity of bactericidal action was not demonstrated to any marked degree. The material was soluble in water and its aqueous solutions had low surface tensions. It was more rapid in its action against *B. subtilis* when the reaction was distinctly alkaline, rather than acid or neutral.

Krog and Marshall [33] found that alkyl-dimethyl-benzyl ammonium chloride exerted a definite bactericidal action on the flora associated with milk-handling equipment and that it is stable when used to sanitize properly cleaned pasturizing equipment. Frayer [14] investigated the same compound and noted that it is a highly effective bactericide which is quick acting in the higher and progressively active in the lower concentrations as the time interval is increased. Additional studies on a number of the quaternaries have shown their efficiencies as bactericides and indicated their applications in various fields.

In investigating the effect of one of the compounds on milk, DuBois and Dibblee [34] concluded that it lacks any influence on the bacterial counts of raw or pasteurized milk over a rather wide concentration range. The higher concentrations had an inhibitory effect on growth

of gram-positive, acid-producing organisms but not on that of gram-negative organisms.

Crocker [35] reported that *Ser. marcescens* could be made to grow in broth to which nearly 1,200 ppm of quaternary had been added; this degree of induced resistance was developed in 10 transfers. Five strains of *E. coli* survived 7.7 ppm of quaternary in broth and developed a resistance to 25 ppm, or more, in 6 transfers; 1 strain survived in broth containing 100 ppm. As the resistance of some strains of *Ser. marcescens* to quaternary increased, there was a corresponding reduction in pigment production and some strains were completely nonpigmented. As *E. coli* developed resistance, it lost its ability to produce gas and certain other characteristics.

The quaternaries are affected less by organic matter than are the chlorine compounds. This is a distinct advantage. They are active in neutral or alkaline solution. This sometimes is an advantage. Botwright [36] emphasized that bactericidal rinses often follow cleaning solutions in which alkaline detergents are used so that compounds active in the alkaline range are desirable. The quaternaries are not compatible with some of the cleaning materials used, such as soaps, hexametaphosphate, certain wetting agents, etc.

The range of possibilities in connection with the chemical structure of the quaternaries that could be prepared suggests that compounds with somewhat different properties for somewhat different purposes can be developed. Studies on the effect of constitution on the bactericidal properties of the compounds already indicate progress along this general line.[37]

Effect of Water Supply on Activity of Quaternaries. Certain water supplies interfere with the bactericidal activity of quaternary ammonium compounds. Shere [38] reported that, to kill bacteria in water of 400 ppm hardness, quaternaries required from 2 to 400 times the concentration required in distilled water, while sodium hypochlorite was unaffected. Mueller and Seeley [39] found no close correlation between water hardness, as measured by standard soap titration, and the bactericidal activity of a quaternary. Calcium, magnesium, and ferric iron decreased the activity of the quaternary while potassium, sodium, and lithium had no adverse effect. Ferric iron was more detrimental than calcium or magnesium. When water contained as much as 1,000 ppm of calcium or magnesium, 200 ppm of quaternary was sufficient to give approximately 100% kill of *E. coli* with 8 min contact, whereas as little as 10 ppm of ferric iron completely inactivated the quaternary. Chambers *et al.*[40] noted that waters containing bicarbonates, sulfates,

and chlorides of calcium and/or magnesium cause the most interference with the bactericidal action of quaternaries. Ferrous bicarbonate interfered slightly, but its interference was minimized by oxidation of the iron. Water with a mineral content of mostly sodium or potassium bicarbonate did not interfere seriously with the bactericidal action. When such water was boiled, most of the bicarbonates were converted to carbonates with no apparent change in interference. Lime, or partial zeolite softening, removed much of the interference from a hard water. Tetrasodium pyrophosphate and the tetrasodium salt of ethylenediamine tetraacetic acid also reduced the interference sufficiently to offer promise in practical application.

Quaternary Detergent-sanitizers. Humphreys and Johns [41] stated that in most commercial quaternary detergent-sanitizers a quaternary ammonium compound is combined with a nonionic wetting agent, alkaline detergent, and one of the polyphosphates, thus permitting cleaning and bactericidal treatment in a single operation. Mueller [42] found that the bactericidal properties of quaternaries are increased in detergent-sanitizers because of the relatively high *p*H. Also, properly formulated detergent-sanitizers contain sequestering agents for calcium and magnesium which are known to decrease the bactericidal efficiency of quaternaries. Seven detergent-sanitizers tested by Barber [43] showed considerable variation when used in water of 51 ppm hardness and containing 0.5% ice cream mix. The variations were greater in water of 514 ppm hardness. A comparative study of a detergent-sanitizer and a hypochlorite indicated that the detergent-sanitizer was more effective against a gram-positive than against a gram-negative organism, whereas with the hypochlorite the reverse was true. Elliker *et al.*[44] found that satisfactory sanitization of milking equipment could be accomplished by the use of either a detergent-sanitizer or an alkaline cleaner plus a bactericidal rinse with hypochlorite. The detergent-sanitizer exhibited marked specificity against thermoduric bacteria and no accumulation of *Pseudomonas* species occurred.

Iodophors. Certain surface-active nonionic synthetic detergents take up iodine and hold it in loose chemical combination. The combination of iodine and carrier is called an iodophor. The carrier provides a solubilizing medium for the iodine and modifies its properties. Iodophors are effective bactericides and are capable of considerable dilution without precipitation of the iodine. They are more bactericidal than equivalent solutions of iodine in alcohol or potas-

sium iodide. Formulations containing iodine are designed for use as bactericides or detergent-sanitizers.

Johns,[45] employing *M. pyogenes* var. *aureus*, *E. coli*, *Pseudomonas aeruginosa*, and *B. subtilis* as test organisms, found that iodophors were at least as effective as chlorine or quaternaries when used at 68 and 113 F. Addition of 0.5% skim milk had less effect on bacterial destruction by iodophors than by chlorine or quaternaries. Mueller[46] reported that 2 iodine detergent-sanitizers, at a concentration of 25 ppm available iodine, gave about the same bacterial destruction as 100 ppm available chlorine, in the form of calcium hypochlorite, when 4 nonspore-forming organisms were used as test cultures and when comparisons were made in the presence of hard water, milk, or dishwash soil. Both preparations at a concentration of 12.5 ppm available iodine were equal to 100 ppm available chlorine when tested against *E. coli* and *Salmonella typhosa*. However, with *Ps. aeruginosa* and *M. pyogenes* var. *aureus*, 12.5 ppm available iodine was equivalent to 100 ppm available chlorine in distilled water but not in the presence of milk or dishwash soil.

Johns and Berzins[47] noted that an off flavor could be detected in milk when 8 ppm of one iodine compound or 16 ppm of another was added to milk. Greater amounts gave increasingly bitter and unpleasant flavors.

MILK PRESERVATIVES

Milk preservatives, some of which were sold under trade names, at one time were used rather widely, but regulations prohibiting the practice largely have eliminated it. At present, ice and refrigeration machinery provide adequate facilities for holding milk and cream so there is no longer need to use preservatives, except in samples intended for special purposes.

General Effect on Organisms in Milk

Action of a preservative on the organisms in milk and cream depends on the amount used. A given concentration may simply prevent growth or decrease its rate, whereas a higher concentration may destroy organisms. The period a given preservative in a certain concentration will prevent changes in milk varies a great deal and depends on the species present and the temperature. A preservative commonly acts differently on different organisms and, as a result, the proportion of the various species present in a lot of milk definitely

may be modified. This is objectionable if the acid-producing organisms are prevented from growing and those attacking protein are not so effectively checked. In general, the lower the temperature of holding milk or cream containing a preservative, the longer the organisms are restrained.

Preservatives That Have Been Used in Milk

The more important preservatives that were at one time used in milk intended for consumption are formaldehyde, boric acid, borax, benzoic acid, and salicylic acid. In suitable concentrations these compounds definitely delay growth of organisms. Various carbonates also have been added to milk but their main effect is to neutralize the acid produced, rather than prevent growth of organisms.

Formaldehyde. Formaldehyde is very effective in limiting growth of organisms in milk and its action is illustrated by the work of Chester and Brown.[48] The investigators found that when the concentration of formaldehyde in milk was from 1 to 800 to 1 to 2,000 there was a rapid decrease in bacteria during the first 24 hr and then a slow decrease, until at the end of 5 days the milk was practically sterile or contained only resistant spores. With a proportion of 1 to 5,000 there was rapid reduction in the bacteria for 4 to 6 hr and then a slower decrease, after which multiplication began. A ratio of 1 to 10,000 gave, in some cases, a slight decrease before the increase started, whereas 1 to 20,000 simply delayed the increase. With a proportion of 1 to 40,000 the increase in bacteria was rapid from the first but less rapid than without formaldehyde and the milk kept two to three times as long as untreated milk. *S. lactis* appeared to develop better in the presence of formaldehyde than the other bacteria ordinarily found in milk.

Hydrogen Peroxide. Use of hydrogen peroxide in combination with heat was introduced as a method of treating milk by Budde and the product was called Buddeized milk. The milk was heated to 122 F and enough hydrogen peroxide added to give a concentration of 0.03 to 0.035%. After being stirred for 15 to 30 min, the milk was drawn into bottles, tightly stoppered, the bottles held in a water bath at 122 F for 2 to 3 hr, and then cooled. It appears that pathogens may not be destroyed by this method. An excess of hydrogen peroxide gives an off flavor to the milk.

There are differences among organisms in their resistance to hydrogen peroxide. Nambudripad and Iya [49] found that the time required to destroy various organisms with 500 ppm (by weight) of hydrogen

peroxide at 98.6 F ranged from 40 min with *Alc. viscosus* to 1,080 min with a *Bacillus* species. Catalase-producing organisms were destroyed readily. Giolitti [50] noted that *E. coli* and *Brucella abortus* were destroyed much more quickly by hydrogen peroxide than *M. tuberculosis.*

Morris [51] reported that treatment of milk with 0.2% hydrogen peroxide, followed, after a period, by the addition of catalase to remove any residual hydrogen peroxide, compared favorably with pasteurization in bacterial destruction. Anaerobic spore-forming organisms were eliminated by the peroxide treatment but not by pasteurization. Oxidized flavors developed in 70% of the pasteurized samples but in none of the peroxide-treated samples. The flavor of the peroxide-treated milk was satisfactory except for a slight malty flavor.

Chlorine Compounds. The efficiency of chlorine compounds for the bactericidal treatment of dairy equipment has led to studies on their action in milk. Most investigators have found that they destroy relatively few organisms. Zoller and Eaton [52] noted that sodium hypochlorite has a relatively low bactericidal effect in milk, considering its vigorous action on bacterial cells. Only when there was an excess of the hypochlorite present, as indicated by tests, did they obtain more than a 50% reduction in plate counts, and then the highest reduction was 80.8%. Prucha [53] reported that large amounts of chemical bactericides are required to produce any effect on bacteria in milk, and that milk will acquire an off flavor and be spoiled before the bacteria in it are affected. Young [54] found that preparations containing sodium hypochlorite did not materially reduce the bacterial count on milk when used in concentrations as high as 200 ppm available chlorine. An odor and taste could be detected in concentrations of 50 ppm and were noticeable in concentrations of 100 ppm. He concluded that the unpleasant odor and taste produced when sodium hypochlorite is added to milk in sufficient quantities to act as a preservative render its use impractical. Johns [55] reported that formaldehyde was a much more effective milk preservative than 2 hypochlorite and 2 chloramine-T products studied. When added to freshly drawn milk, which was cooled quickly, in the maximum concentrations not detectable by taste after 18 hr at less than 60 F, the preservative effect of each of the chlorine compounds was relatively slight; if the milk was not promptly cooled the effect was more noticeable, yet the bacterial count increased so greatly as to preclude the possibility of chlorine preservatives replacing prompt and thorough cooling. Hauser and King [56] found that addition of hypochlorite to fresh milk

may appreciably reduce the bacterial content whether or not there is subsequent pasteurization and that hypochlorites added to milk in amounts not readily detectable by taste may produce a mild but definite preservative effect.

Antibiotics. The preservative effect of various antibiotics on milk has been studied by several investigators. With certain of the antibiotics, results obtained are variable, due presumably to differences in bacterial floras of the samples tested and the specificity of the antibiotic.

Penicillin. Foley and Byrne [57] determined the effect of adding 3 units of pencillin per ml on the plate count of good- and poor-quality raw and pasteurized milk. They concluded that such an addition to good-quality milk held at low temperatures is of little benefit because bacterial growth takes place slowly and penicillin is active mainly against growing organisms. Such an addition might be of value if the milk were not cooled properly. Penicillin was more effective in pasteurized than raw milk, due to the presence of penicillin-resistant, gram-negative organisms in raw milk. Addition of penicillin to milk just prior to pasteurization gave milk with a lower bacterial count than was obtained by pasteurization alone. Wilkowske and Krienke [58] noted marked retardation of bacterial growth for about 72 hr in raw milk containing 1 unit, or more, of penicillin per ml and held at 50 F. Stoltz and Hankinson [59] added penicillin to raw milk, held the samples at 44.6 F, and determined the increase in bacteria. Milk containing 0.01, 0.1, or 1.0 unit per ml did not increase in count as rapidly as the control sample and the increase was slower with the larger amounts of penicillin. Shiveler and Weiser [60] noted that the bacterial population attained by incubating raw milk for 24 hr at 68 F was reached by a sample of the same milk containing 5 units of penicillin per ml in 72 hr when held under the same conditions.

Aureomycin. The addition of 0.1 to 0.5 ppm aureomycin to raw milk held at 44.6 F has a restraining action on bacterial multiplication.[59] The restraining action is very marked for 36 hr but not for 48 hr. Aureomycin is very effective against the bacterial flora of pasteurized milk.[60]

Streptomycin. No appreciable inhibition of bacteria was noted when 10 ppm of streptomycin was added to raw milk and the milk held at 68 F.[60] Addition of 5,000 ppm gave pronounced inhibition for >48 hr when the milk was held at 44.6 F; 100 ppm gave pronounced inhibition for 36 hr.[59] Poetschke [61] found that addition of 2 mg of streptomycin per 100 ml of human milk is an effective pre-

servative. This method of preservation was used for 1 yr with the human milk bank at a clinic. Streptomycin did not impair the nutritional or physiological qualities of the milk for infant feeding and caused no harmful effects. Nagel [62] reported that low concentrations of streptomycin destroyed organisms more readily in human milk than in cows' milk. *Salmonella* species were more resistant to streptomycin than *E. coli.*

Preservation of Milk and Cream for Analytical Purposes

Milk and cream intended for analytical purposes can be treated with a preservative that will not interfere with the analyses to be made. Samples for fat determinations often must be held for extended periods at temperatures favorable for growth, and bichloride of mercury [63] is frequently used for their preservation because of its toxic effect on all types of micro-organisms. The chemical is available in tablets which also contain some coloring material so that the preserved milk or cream will have a very abnormal appearance and not be consumed accidentally. When a preservative is used with milk or cream samples, it must be well distributed if growth of organisms is to be prevented in all parts of a sample. Molds sometimes develop on samples preserved with bichloride of mercury because the reagent is not thoroughly distributed.

An organic mercury compound, called "ortho-mercurate," has been recommended for preserving composite milk samples for butterfat testing. The compound is stated to be nonpoisonous and does not retard the dissolution of casein by sulfuric acid.[64] Milk samples containing the preservative were held at 90 F for 30 days without spoilage or fat deterioration. Tablets containing potassium dichromate also are being distributed for use as a preservative for composite milk samples. Wegener [65] found that addition of 0.2% potassium dichromate to milk was sufficient to prevent the development of acid.

REFERENCES

1. *Food Inds.*, **21** (3), 295 (1949).
2. *Milk Plant Monthly*, **36** (11), 22 (1947).
3. *Ind. Eng. Chem.*, **19**, 1338 (1927).
4. *Ind. Eng. Chem.*, **20**, 63 (1928).
5. *Ind. Eng. Chem.*, **20**, 179 (1928).
6. *Ind. Eng. Chem.*, **20**, 378 (1928).
7. *J. Agr. Research*, **38**, 521 (1929).
8. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 867 (1950).
9. *J. Milk and Food Technol.*, **10**, 257 (1947).
10. *Yale J. Biol. and Med.*, **11**, 33 (1938).
11. *Proc. Soc. Exptl. Biol. Med.*, **40**, 86 (1939).

12. *Abstr. Dairy Technol. Conference, Ohio State Univ.*, 29 (1948).
13. *Food Inds.*, **21** (2), 82 (1949).
14. *Vt. Agr. Expt. Sta. Bull.* 511 (1944).
15. *J. Milk and Food Technol.*, **16**, 61 (1953).
16. *Ia. Eng. Expt. Sta. Bull.* 150 (1941).
17. *Ia. Eng. Expt. Sta. Bull.* 132 (1937).
18. *Sci. Agr.*, **14**, 585 (1934).
19. *Public Health Repts.*, **65**, 503 (1950).
20. *Public Health Repts.*, **63**, 934 (1948).
21. *Ice Cream Rev.*, **11**, 86 (Feb. 1928).
22. *J. Dairy Research*, **16**, 327 (1949).
23. *Sci. Agr.*, **10**, 553 (1930).
24. *Sci. Agr.*, **12**, 38 (1931).
25. *Vt. Agr. Expt. Sta. Bull.* 369 (1934).
26. *J. Bacteriol.*, **15**, 189 (1928).
27. *J. Bacteriol.*, **32**, 57 (1936).
28. *J. Dairy Research*, **16**, 23 (1949).
29. *J. Milk and Food Technol.*, **15**, 285 (1952)
30. *Biochem. J.*, **41**, 337 (1947).
31. *J. Milk and Food Technol.*, **17**, 136 (1954).
32. *Am. J. Hyg.*, **26**, 46 (1937).
33. *J. Milk Technol.*, **5**, 343 (1942).
34. *J. Milk Technol.*, **9**, 260 (1946).
35. *J. Milk and Food Technol.*, **14**, 138 (1951).
36. *J. Milk Technol.*, **9**, 101 (1946).
37. *J. Bacteriol.*, **49**, 277 (1945).
38. *Milk Plant Monthly*, **37**, 66 (Mar. 1948).
39. *J. Dairy Sci.*, **31**, 723 (1948).
40. *Public Health Repts.*, **70**, 545 (1955).
41. *J. Milk and Food Technol.*, **16**, 186 (1953).
42. *J. Milk and Food Technol.*, **12**, 240 (1949).
43. *J. Milk and Food Technol.*, **12**, 257 (1949).
44. *J. Milk and Food Technol.*, **13**, 215 (1950).
45. *Can. Dairy Ice Cream J.*, **33** (2), 31 (1954).
46. *J. Milk and Food Technol.*, **18**, 144 (1955).
47. *J. Milk and Food Technol.*, **17**, 313 (1954).
48. *Del. Agr. Expt. Sta. Bull.* 71 (1905).
49. *Indian J. Dairy Sci.*, **4**, 38 (1951).
50. *Atti soc. ital. sci. vet.*, **1**, 190 (1947).
51. *Proc. Western Div. Am. Dairy Sci. Assoc.* 130 (1950).
52. *J. Dairy Sci.*, **6**, 131 (1923).
53. *Ill. Agr. Expt. Sta. Circ.* 332 (1929).
54. *Am. J. Public Health*, **20**, 308 (1930).
55. *Can. Public Health J.*, **21**, 163 (1930).
56. *J. Assoc. Offic. Agr. Chemists*, **28**, 417 (1945).
57. *J. Milk and Food Technol.*, **13**, 170 (1950).
58. *J. Milk and Food Technol.*, **14**, 92 (1951).
59. *J. Milk and Food Technol.*, **16**, 157 (1953).
60. *J. Milk and Food Technol.*, **16**, 125 (1953).
61. *Monatsschr. Kinderheilk.*, **98**, 177 (1950).
62. *Arztl. Wochenschr.*, **5**, 393 (1950).
63. *J. Dairy Sci.*, **2**, 170 (1919).
64. *Milk Plant Monthly*, **39** (9), 89 (1950).
65. *Lebensmitteltierarzt*, **3**, 107 (1952).

Growth of Bacteria in Raw Milk

6

The numbers of bacteria that gain entrance to milk during production often are increased, sometimes greatly so, through multiplication during holding, and the excessive counts on certain lots of milk reaching dairy plants are due primarily to growth. The rate at which organisms increase in milk is determined primarily by two factors, temperature of holding and species present. The holding temperature also greatly influences the types of organisms that grow, since the maximum, optimum, and minimum growth temperatures vary widely with different species; even within a species there may be variations in the growth temperatures of different strains.

Control of Growth. Bacterial growth in milk on farms and during transportation to plants is controlled primarily by lowering the temperature. Even a moderate drop is helpful, and on most milk-producing farms an attempt is made to cool the freshly drawn milk. Because of inadequate or improperly used facilities, the temperature drop often is less than is desirable and significant bacterial growth occurs.

Changes in Numbers of Bacteria at Some Common Holding Temperatures. The increases that occur in numbers of organisms in milk at 40, 50, and 60 F are illustrated by the accompanying data reported by Ayers *et al.*[1] as averages of a number of samples.

Changes in Numbers of Bacteria in Milk at Various Temperatures

Cows Clean and Bedded, Small Top Pails, Sterile Utensils
(Average 20 Samples)

Temperature of Holding	Bacteria per Milliliter				
	Fresh	24 Hr	48 Hr	72 Hr	96 Hr
40 F	4,295	4,138	4,566	8,427	19,693
50 F	4,295	13,961	127,727	5,725,277	39,490,625
60 F	4,295	1,587,333	33,011,111	326,500,000	962,785,714

Cows Dirty, Manure Removed Twice Weekly, Utensils Not Sterile
(Average 30 Samples)

Temperature of Holding	Bacteria per Milliliter				
	Fresh	24 Hr	48 Hr	72 Hr	96 Hr
40 F	136,533	281,646	538,775	749,030	852,835
50 F	136,533	1,170,546	13,662,115	25,687,541	41,207,272
60 F	136,533	24,673,571	639,884,615	2,407,083,333	5,346,666,666

From the results it is evident that at 40 F growth is comparatively slow, at 50 F it is considerably more rapid, and that the difference between 50 and 60 F is very striking. The data further indicate that rate of multiplication of bacteria is influenced by the quality of the milk and that the increase in organisms percentage-wise is less in clean milk than in higher count milk. This is to be expected since, with clean milk, a considerable portion of the organisms come from the interior of the udder and are species that grow comparatively slowly; with higher count milk, many of the organisms come from external sources and include species that multiply rapidly. This difference between clean and higher count milk explains why conditions that are reasonably satisfactory in preventing changes in certain lots of milk are unsatisfactory for other lots and also why conditions of production have a greater influence on the quality of milk after holding than might be expected from the actual number of bacteria introduced by the careless methods.

In general, over the range from 50 to 70 F, small temperature differences have a striking effect on the rate of bacterial growth in milk and, accordingly, on the time of appearance of changes in it. This range is involved primarily on farms where cooling facilities are inadequate or not properly used. Milk showing changes due to bacterial growth when received at dairy plants often has been held well up in this range, as usually is evident from its temperature on arrival.

Influence of Temperature on Fermentation. Besides influencing the rate of bacterial growth in milk, the temperature of holding defi-

nitely influences the type of fermentation that occurs. Lactic acid streptococci grow actively over a rather wide temperature range (70 to 98.6 F), and in this range souring is the conspicuous change in milk. At both higher and lower temperatures other species become more important. At higher temperatures gas formation sometimes is conspicuous and at lower temperatures off flavors and odors develop due to bacterial action on protein and fat.

The temperature at which a particular fermentation is noted under practical conditions may not be the most favorable for the causative organism but, because of the restraining action on competing species, it is comparatively very satisfactory; for example, *Alc. viscosus* grows more rapidly at 70 F than at 50 F but in raw milk at 70 F *S. lactis* is so active that *Alc. viscosus* rarely produces ropiness, whereas at 50 F it finds much less competition.

Cooling and Holding Milk on Farms. Holding milk for varying periods is necessary on practically all dairy farms. Although it is most satisfactorily accomplished by entirely preventing growth of organisms, this is not always possible because the low temperatures required are not available and a limited amount of growth often is accepted in practice. Adequate holding methods involve two steps, (1) reasonably rapid cooling to suitable temperatures, and (2) maintenance of such temperatures during the holding period. Because of the increasing use of every-other-day pickup of milk during all or part of the year, holding periods now often are longer than formerly and this necessitates cooling to lower temperatures.

Cooling in Water. In many important milk-producing areas, well water is used extensively to cool milk by holding cans of milk in a tank of it and sometimes also by using it in a milk cooler to lower the temperature quickly. It is convenient and inexpensive. Although many farms cool and hold milk satisfactorily with well water, attempts along this line may fail, especially during warm weather, because the water is not cold enough or insufficient amounts are used. Adequate springs for cooling milk are rare. Ice is inconvenient and often expensive.

The value of mechanical refrigeration in maintaining tanks of cooling water at low temperatures and/or in operating milk coolers now generally is recognized as a result of many investigations. Undoubtedly, such refrigeration has been a big factor in the improvement in milk quality during recent years. Without it, many areas would find it difficult to provide adequate supplies of high-quality milk.

The rate of cooling of cans of milk in water has been studied ex-

tensively. Price *et al.*[2] noted that, in water at 35 to 40 F, milk in 10-gal cans cooled from 95 to 60 F, or below, in 1 hr and to 56 F in 2 hr; when the milk was precooled with a tubular surface cooler to 67 F, it cooled to 55 F in 1 hr and to 52 F in 2 hr in the water. With water at 35 F circulated around the cans, milk in 10-gal cans cooled from 95 F to below 50 F in 1 hr and to 42 F in 2 hr; when the milk was precooled with a tubular cooler to 67 F, it cooled to 44 F in 1 hr and to 42 F in 2 hr in the circulating water. The bacterial content of milk held in 10-gal cans in water at 35 to 40 F did not increase during the first 12 hr. Stirring milk in 10-gal cans in water at 35 to 40 F did not materially increase the rate of cooling.

Marquardt and Dahlberg[3] found that, when milk above 90 F was placed in water at 35 to 40 F and stirred after 1 and 2 hr, the temperature was around 60 F in 1 hr and at 50 F in 2 hr; with the milk at 70 to 80 F as it was placed in the tank, the temperature dropped to 50 F within 1 hr. Thermometers set in fixed positions in an unstirred can of milk and read at 10-min intervals for 4 hr indicated that the milk cooled rapidly at the sides and in the center at a point near the handles and, presumably, more rapidly below this; however, 1 in. below the neck and in the center of the can, cooling was so slow the milk reached 60 F only after 4 hr.

According to Anderson,[4] cooling milk in a tank with water above 50 F and without agitation of the water did not afford adequate protection against bacterial growth; he concluded that fresh milk should be cooled entirely and quickly to below 50 F, preferably to 40 F, to prevent growth. Anderson and Nicholas[5] reported that cooling milk with well or spring water in a tank without agitation of the water is not satisfactory for production of a high-quality product unless the water is approximately 50 F, or lower, and unless that temperature is maintained during the entire cooling period; they suggested running water should maintain a temperature of 48 F, or below, to cool fresh milk efficiently.

Nicholas and Blasingame[6] noted that milk will cool more rapidly and uniformly in farm electric coolers when the water bath is agitated and that the agitation should continue for at least 1 hr. They found a can of milk will cool more in the first hour than in the 11 succeeding hours. Under the same thermostat limits, milk will cool more rapidly with a water to milk ratio of 5 than with one of 3.

Abraham and Outwater[7] concluded that mechanical refrigerators are the most effective means of cooling milk on farms and probably the most economical, except where an adequate supply of cold spring

water at 50 F, or less, is readily available, and also that unless a producer is equipped with a thermostatically controlled electric pump and has an ample supply of well water at 50 F, or lower, use of well water alone is not dependable in most cases.

A study of the cooling rate of milk in various types of mechanical farm milk coolers, using thermocouples located in various positions in the cans, was carried out by Shipman.[8] A tank-type cooler which formed no ice bank was the least effective type examined, either with 1-hr agitation or without agitation of the water; 8 to 10 hr were required to cool the milk at the top of the can to 50 F. A tank-type cooler forming a heavy ice bank and provided with water agitation for 2.75 hr cooled milk at both the top and bottom of the can to below 40 F in less than 2 hr; a similar cooler without water agitation required about 8 hr to attain this result. A spray-type cooler, with water flowing over the tops of the cans, cooled milk at the top of the can to 47 F and at the bottom of the can to 42 F in 1 hr. At all temperatures above 39 F, milk in cans was warmer at the top than at the bottom, whereas the opposite was true of milk in cans cooled to below 39 F.

Cooling in Air. In some instances attempts are made to cool milk by exposing cans of it to cold air, for example, out-of-doors or in an unheated room in winter, or in a refrigerated room. Air conducts heat from the cans only very slowly and adequate temperature drops are greatly delayed. Roberts and Larson[9] reported that after 8 hr in a dry box cooler at 41 F, milk in the tops of 10-gal cans was above 70 F; they concluded that cold air is practically worthless for cooling milk in 10-gal cans.

When cold air is in active motion, its cooling effect definitely is greater than when it is quiet. Although exposure of milk in cans to cold air in active motion results in slow cooling, exposure for extended periods may result in freezing. Because of this danger, producers in certain areas do not take advantage of the natural refrigeration available and in winter even hold milk in a comparatively warm place to insure against freezing.

The temperature of properly cooled milk is maintained readily in a refrigerated room, and such rooms for the storage of various products are an important part of all modern dairy plants.

Farm Bulk Milk Tanks. Bulk milk tanks are very satisfactory for holding milk on farms (see p. 150). When operating properly, the desired milk temperature can be obtained quickly and bacterial growth prevented for any reasonable period.

Marth *et al.*[10] reported that one of the bulk holding tanks studied by them cooled milk to 38 F as rapidly as 3 milking machines supplied it; the tank had a capacity of 220 gal and a 2 hp motor operated the compressor. A 150-gal tank, with a compressor operated by a 0.75 hp motor, cooled milk to 38 F but at a slower rate than the larger tank.

Delivery of Uncooled Milk. Macy[11] studied, under summer conditions, the growth of bacteria in uncooled morning milk from the time of production until delivery at the pasteurizing plant and noted regular increases although samples with low initial counts also had low final counts. He concluded that in areas where conditions can be controlled satisfactorily the farmer might be permitted to deliver uncooled morning milk to the pasteurizing plant but that, as a general practice in all communities, it is not to be recommended.

Various market milk regulations permit the delivery of uncooled milk under certain conditions. Usually, the milk must be delivered within 2 hr of the completion of milking to the plant or receiving station where it can be processed or cooled promptly.

With twice-a-day delivery of milk for manufacturing purposes, there sometimes is little or no cooling. Producers and receivers may fail to recognize that with long truck routes, etc., 4, 6, or even 8 hr may elapse between milking and delivery of the milk and that without cooling of the product there may be extensive growth of bacteria in it. The interval between milking and cooling or processing of the milk is the important consideration, not the number of deliveries per day.

General Considerations. Proper cooling and holding of milk on farms frequently is neglected unless plant standards are high and rigidly enforced. Many milk producers fail to suspect extensive bacterial growth unless the product actually is curdled, and this may lead to increased carelessness in the cooling and holding unless complaints come from the plant.

When evening milk is held overnight on the farm and delivered with the fresh morning milk, it often has a higher bacterial count than the morning milk and, in some instances, the difference is striking. That the large numbers of bacteria in many lots of milk held on farms are due to slow and/or incomplete cooling is indicated by the temperature of the milk at delivery and by the fact that, with proper cooling, evening milk is no higher in bacteria at delivery than fresh morning milk. Dearstyne and Jones[12] found great variations in the bacterial contents of evening and morning milk supplied to various

dealers and pointed out the importance of this in grading milk on the basis of numbers of contained bacteria.

Effect of Delayed Cooling. From studies on the influence of delayed cooling on the quality of milk, Frayer[13] concluded that prompt cooling to 50 F, or less, is a vital factor in producing good-quality milk which will keep well and that damage to the future quality of milk is roughly proportional to the period elapsing before cooling. He[14] later reported that cooling milk immediately to 50 F is a fairly satisfactory procedure and that cooling morning milk to 60 F is fairly satisfactory, but that cooling milk to be held overnight on the farm to this temperature is unsatisfactory. Cooling milk immediately to 40 F, or lower, and holding it there seemed to produce the best results in respect to its quality, both present and future. Fresh milk that was not cooled deteriorated progressively for each hour of such delay and the lower the initial temperature the better. In studies on pure cultures inoculated into milk at 75 F, Frayer[15] found that the effect of delayed cooling varied widely with different species. The various delays before the milk was cooled gave average increases in bacterial counts as follows: 2 hr, 22%; 4 hr, 221%; 6 hr, 1,230%; 8 hr, 6,229%.

Concentration of Bacteria in Fat Layer. When milk is allowed to stand, the fat concentrates in the surface layers and brings with it many of the bacteria so that the numbers per milliliter definitely are larger than in the lower layers. This is shown by the accompanying data reported by Marquardt and Dahlberg.[3]

Concentration of Bacteria and Fat in Top of Milk on Holding at 50 F

	Bacteria per Milliliter	Per Cent Fat
Fresh milk mixed	53,000	5.0
Top milk 1 hr old	108,000	7.7
Top milk 2 hr old	167,000	15.3
Top milk 5 hr old	262,000	16.4
Mixed milk 6 hr old	53,000	5.0

Anderson[4] noted that in the majority of instances 90%, or more, of the organisms in a 10-gal can of milk cooled 12 hr are in the cream layer. Nicholas and Anderson[16] found the ratio between the number of organisms in fresh milk and in the cream layer of cooled milk varied from as low as 1 to 5.5 to as high as 1 to 173.

With adequate cooling of milk being held on farms, concentration of bacteria in the cream layer probably is of no practical significance. When the milk is mixed, they are redistributed to give the product

essentially the same count as it had originally. Without adequate cooling, concentration of organisms in the cream layer may result in more rapid changes there than in the lower layers. For example, ropiness in the cream layer of a can of milk apparently is influenced by the concentration of organisms there, although such factors as air supply also may be involved.

The cream on a can of milk standing in cooling water sometimes is considerably higher in temperature than milk well down in the can. Various factors may be responsible, including too low a water level for the height of the can. Under such conditions, opportunities for growth of the bacterial population in the cream are especially good. Moseley [17] reported the following temperatures in the cream layers and in the milk at bottoms of cans in mechanical coolers on farms.

Temperatures in Cream Layers and Milk at Bottoms of Cans in Mechanical Coolers on Farms

Producer	Temperature (F)		
	In Cream Layer	At Bottom of Can	Of Cooling Water
1	84	45	44
2	69	44	44
3	65	42	41
4	48	42	42
5	70	44	44
6	44	44	44
7	79	46	46
8	62	43	43
9	79	44	43
10	82	47	46
11	68	44	44

Transportation of Milk from Farms. Transportation of milk in cans from farms to receiving stations or plants involves a number of considerations. Open trucks, or trucks covered only with a tarpaulin, offer little protection against collection of dust, insects, and other material on can lids, lips, etc., from which they may fall into the milk on dumping; also, there is little protection against temperature changes. Properly closed trucks protect against the collection of extraneous matter on cans and, if insulated, against both the heat of summer and freezing winter temperatures. Closed trucks may delay loading and unloading, particularly if large loads are being hauled, but they are essential to adequate handling of milk in cans and are being demanded by more and more regulatory officials. From the

standpoint of adequate protection, the bulk handling of milk has definite advantages.

Poorly cooled or uncooled cans of milk may significantly increase the temperature of cans of well-cooled milk near them. Anderson [4] found that, during a transportation period of about 2 hr, cooled milk at 38.1 to 46 F in cans protected only by a tarpaulin showed temperature increases of 12.9 to 21.5 F. The increases were dependent on two factors, air temperatures of 59.8 to 70.8 F and proximity of warm cans of morning milk.

BACTERICIDAL ACTION OF MILK

For a period after milk is drawn there may be a decrease in the number of bacteria in it, as determined by the plate method, and this ordinarily is considered to be due to a bactericidal property. Blood and body fluids in general have the ability to destroy many species of organisms and some such action would be expected with milk. The bactericidal action of milk is weak and perhaps should be looked upon as a restraining or antiseptic action rather than one actually destroying organisms, although with certain species it may be very effective. It first was detected by Fokker [18] in an investigation of goats' milk. With inoculated raw milk he observed slower souring and a smaller number of colonies on plates than with heated milk similarly inoculated. Various investigators have found that, when pure cultures of bacteria are added to raw milk and the numbers determined after varying periods, very definite decreases sometimes are obtained. With this procedure, inoculation of fairly large numbers of bacteria into milk may give a more decided decrease than inoculation of only small numbers.[19]

Variations in Bactericidal Action. Early in the investigations on the bactericidal property of milk Hunziker [20] pointed out that the degree of the action varies greatly in milk of different animals and sometimes in milk of the same animal at different milkings. Variation in the milk of individual animals also was noted by Chambers,[21] who reported that in a group of 11 cows the milk of only 3 possessed bactericidal action. Jones [22] observed that the inhibitory substance varies in the milk from different quarters of the same cow and that it is present in the milk of the first days of lactation as well as later. Hanssen [23] found the property greatest in summer.

Various bacteria are influenced very differently by the bactericidal action of milk and, accordingly, this property must be considered as

more or less specific. The extreme variation is illustrated by samples of milk which show pronounced action for one species and none for another. Organisms which grow rapidly in milk appear to be included among the species influenced by the bactericidal property. Certain investigators [19, 24] have noted a slight but definite inhibitory action on *S. lactis*. Chambers,[21] however, reported that the milk of two cows, which was known to possess bactericidal properties, had no action on *S. lactis*. Curran [25] found that an organism capable of sweet curdling milk rapidly was restrained markedly in certain samples of mixed raw milk, and coagulation was delayed from 3.5 to 6 hr in the different samples; *S. lactis* was restrained only slightly. The bactericidal activity was present in the milk over a period of several months and was confined to Jersey milk. Certain coliform strains are actively inhibited by some milk.[26, 27]

Jones [28] noted that addition of milk filtered through a Berkefeld candle V, or heated at 136.4 F for 20 min, to blood agar plates interfered with development of colonies of the scarlet fever *Streptococcus*, the inhibition being proportional to the amount of milk used. When the organism was added to the milk that had been heated and the incubation prolonged, it was killed. Boiling the milk destroyed the inhibitory substance.

Action of Heat on Bactericidal Property. High temperatures destroy the bactericidal property of milk but the data [23, 29] indicate that the temperature required for such destruction varies with different organisms. Freezing temperatures for short periods had no influence on the property, but freezing for long periods decreased it. Jones and Little [30] found that the substance in milk which inhibited a mastitis *Streptococcus* was relatively resistant to heat.

In studies on a bactericidal substance in milk that was specific for certain strains of coliform organisms, Morris [26] found the following effect of heat.

Effect of Heat on a Bactericidal Substance in Milk That Was Specific for Certain Coliform Strains

Culture	Milk Heated 30 Min at	Plate Count per Milliliter: At Inoculation	Plate Count per Milliliter: After 4 Hr at 98.6 F
1	125.6 F	1,328,000	1,000
	127.4	848,000	40,000,000
2	125.6	316,000	31,000
	127.4	640,000	28,000,000

Causes of Decreases in Bacteria in Milk. The action of milk in causing a decrease in the number of bacteria, as determined by the plate method, appears to be dependent on several factors. Certain bacteria apparently find conditions unfavorable and rapidly die. Some of the leucocytes present may retain their ability to ingest bacteria after the milk is drawn, although there seems to be no evidence of this. McEwen and White [31] noted that the temporary rise in cell content of milk when saline solutions or cultures of various streptococci were injected into the udder did not change the bactericidal or bacteriostatic properties of the milk, even when the udder became inflamed and clots formed in the milk.

Certain of the bacteria gaining entrance to milk apparently are clumped by agglutinins and the plate count thus could be decreased. The agglutinins undoubtedly come from the blood of the producing animal and, since variations occur in the agglutinin content of blood, similar variations would be expected in milk; this may be a factor in explaining variations in the bactericidal property of different lots of milk. Clumps of organisms, some of which contain enormous numbers, are common in milk sediments and, although some of these undoubtedly are due to the manner of growth of the particular species, others may be due to agglutination. Chambers [21] found clumping of certain organisms added to milk but no direct relationship between agglutination and bactericidal action. Antibodies other than agglutinins also are present in milk and may be a factor in the bactericidal property of the product.

Morris and Edwards [27] reported that cows' blood serum contained more of the bactericidal substance than raw milk. The minimum concentration of fresh blood serum which would give 99% destruction of coliform organisms was 0.25%; such destruction required 20% raw milk.

In testing the bactericidal activity of fresh raw milk with a mastitis *Streptococcus*, Jones and Little [30] found that milk of all the cows inhibited the organism although the period of inhibition varied. Jones and Simms [32] concluded that the mastitis *Streptococcus* becomes adapted to the inhibitory agent in milk and that sudden growth of the organism is due to this adaptation rather than to the presence of a resistant type of organism in the original culture. Later, these investigators [33] named the bacterial growth inhibitor of milk *lactenin* and studied it in some detail. Lactenic activity of whole milk was not decreased by removal of fat or coagulation with rennet. The inhibitor was stable for 1.5 hr at *p*H 4 or 10 and for longer periods in neutral solutions. It was removed by reagents which precipitated the

proteins of whey. Part of these proteins could be hydrolyzed by tryptic digestion and the resulting split products, together with salts and sugar, removed by dialysis without appreciable loss of lactenic activity. When the material was dialyzed in a concentrating dialyzer and then completely desiccated, it kept for 3 months with practically no loss of activity. The dry preparation was 200 times as active as the original milk when tested with the scarlet fever *Streptococcus.*

Wilson and Rosenblum [34] found that hemolytic streptococci belonging to the several serological groups vary in susceptibility to lactenin. They noted that human and goat milk contains an antistreptococcal principle which appears to be the same as the lactenin of cow milk. Lactenin [35] was found to be reversibly inactivated by exclusion of atmospheric oxygen. It also was inactivated by cysteine, glutathione, and thioglycollic acid.

The complexity of the problem of inhibition of organisms by raw milk was noted by Auclair and Hirsch.[36] They considered that two substances are involved, lactenin 1 and lactenin 2; these were separated by their different concentrations in milk and colostrum and by small differences in heat and hydrogen-ion stability. Lactenin 1 occurs mainly in colostrum and lactenin 2 mainly in milk; inhibition is greatest when the two act simultaneously. The substances appear to be identical with the lactenin studied earlier.[32,35] *Streptococcus pyogenes* was found to be very sensitive to inhibition when grown aerobically in milk containing peptone, but not when grown anaerobically. S. *agalactiae* was sensitive to inhibition anaerobically but addition of peptone enabled it to overcome the inhibition. The lactenins were considered to be bacteriostatic rather than bactericidal since cultures showing no growth after 48 hr revived by out-diluting them with suitable volumes of broth.

Curran [37] studied the comparative bacterial growth in udders of living cows and in udders following death and removal of the blood. Some bacterial species which were present in udders of living cows at a fairly constant level multiplied actively immediately following death of the cows. Rapid-growing, invasive types of streptococci were most affected. In some cases the udder flora did not increase appreciably during post-mortem incubation and then relatively inert, slow-growing micrococci and streptococci usually were involved. The suddenly increased multiplication of the udder flora that frequently attended withdrawal of the blood was interpreted as suggesting that the circulatory system is directly or indirectly associated with formation of the bactericidal substance in milk.

In studies on the bactericidal property of milk, the effect of various

factors, including associative action, on the growth of organisms should be recognized. It appears that in some instances effects attributed to the bactericidal property of milk may have been due to other causes.

Significance of Bactericidal Property. Although the bactericidal property of milk inhibits growth of certain organisms, it is of little practical importance in the holding of milk unless a low temperature is used. Trout *et al.*[38] noted that milk cooled promptly to 40 F and stored there for 10 hr retained in large part its bacteria-retarding action, whereas this property was virtually gone in milk cooled to and stored at 60 F. Thus the bactericidal action of milk is of value only to milk producers who follow good production practices in cooling milk adequately and promptly.

The bactericidal property of milk may have a significance not yet completely understood. It may protect suckling animals against infection, and it may be a factor in restraining growth of mastitis organisms in the udder. Little[39] reported that in the milk of all cows examined there was present a substance which inhibited growth of mastitis streptococci. Milk of young cows early in their first lactation contained about the same amount as milk from older cows. There was some evidence that the inhibitory substance in milk was more potent against its own homologous organisms than against similar foreign varieties.

PSYCHROPHILIC BACTERIA

In connection with the holding of raw milk at relatively low temperatures, psychrophilic bacteria are of special importance. Their growth temperatures enable them to multiply and even cause spoilage in milk held at temperatures which prevent growth of non-psychrophilic organisms.

Psychrophiles are widely distributed in raw milk. They differ greatly in general characters and belong to various genera. Spore formers appear to be unusual among them.[40] Cultures picked from plates commonly produce various changes in litmus milk, such as proteolysis, alkalinity, and acidity;[41] some cultures are inert.

Sources of the psychrophiles in raw milk are nonsterile farm utensils and equipment, coats of the cows, water supplies, etc. Thomas *et al.*[42] reported that oat grains for cattle feeding contained over 25,000,000 per g. Although psychrophiles are more common in milk with a high, than with a low, initial count, low-count milk sometimes

shows rather rapid increases in count at low temperatures. The difference in growth of psychrophiles in low-count milk is shown by 2 samples held at 40 F at Purdue University. Sample 1 had the following plate counts per milliliter when fresh and after 48, 72, and 96 hr: 3,100; 4,200; 4,500; and 8,000. Sample 2 had corresponding counts of 5,000; 103,000; 420,000; and 1,700,000.

Psychrophiles commonly grow rather slowly when fresh milk is first placed at a low temperature, but later the increase may be quite rapid. In general, multiplication is more rapid at 50 F than at 40 F. This is shown by data obtained at Purdue University on 3 producer samples.

Growth of Bacteria in Three Producer Samples at 40 and 50 F

		Bacteria per Milliliter			
Sample	Temperature of Holding	Fresh	After 24 Hr	After 48 Hr	After 96 Hr
1	40 F	3,100	4,000	4,500	10,000
	50		4,500	18,000	740,000
2	40	16,000	23,000	560,000	1,100,000
	50		62,000	920,000	15,000,000
3	40	28,000	32,000	83,000	810,000
	50		36,000	800,000	59,000,000

Various defects are produced in milk by psychrophiles. Erdman and Thornton [41] reported that all their cultures produced flavor defects in skim milk. Stale and oxidized flavors were noted in 274 (38%) cultures, various sour flavors in 168 (23%), and bitter flavors in 133 (18%); other off flavors noted included incipient rancidity, putrefaction, etc.

In general, psychrophiles are destroyed easily by heat; pasteurization exposures commonly kill them [43] although gram-negative cocci have been reported to survive.[41] Parker *et al.*[44] found that, under conditions of the test, hypochlorites displayed a more rapid bactericidal action against suspensions of representative psychrophilic bacteria isolated from defective milk products than quaternary ammonium compounds.

As improved methods of cooling and holding milk on farms have come into use along with longer holding periods, psychrophiles have assumed more importance. When rapid cooling of milk to low temperatures is introduced on a farm, conditions for multiplication be-

come more favorable for psychrophiles and less favorable for such common types as *S. lactis* so there may be a distinct change in the flora of the milk. The possibility of mesophiles changing their minimum growth temperature with continued exposure to low temperatures may be a factor in building up a psychrophilic flora.[45]

Reduction in the number of psychrophiles in milk delivered to dairy plants requires constant attention to the sources from which psychrophiles enter milk. Holding milk at low temperatures does not replace proper production methods since psychrophiles also can lower the quality of milk.

REFERENCES

1. *U. S. Dept. Agr. Bull.* 642 (1918).
2. *Oreg. Agr. Expt. Sta. Bull.* 268 (1930).
3. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 581 (1930).
4. *Pa. Agr. Expt. Sta. Bull.* 404 (1941).
5. *Pa. Agr. Expt. Sta. Bull.* 454 (1943).
6. *Pa. Agr. Expt. Sta. Bull.* 375 (1939).
7. *J. Milk Technol.*, **7**, 78 (1944).
8. *N. Y. State Assoc. Milk Sanitarians, 21st Ann. Rept.*, 165 (1947).
9. *Kan. Agr. Expt. Sta. Bull.* 295 (1941).
10. *J. Milk and Food Technol.*, **17**, 86 (1954).
11. *J. Dairy Sci.*, **5**, 502 (1922).
12. *J. Dairy Sci.*, **2**, 504 (1919).
13. *Vt. Agr. Expt. Sta. Bull.* 313 (1930).
14. *Vt. Agr. Expt. Sta. Bull.* 343 (1932).
15. *Vt. Agr. Expt. Sta. Bull.* 377 (1934).
16. *Refrig. Eng.*, **42**, 23 (1942).
17. *Milk Ind. Foundation, Conv. Proc.*, **5**, 11 (1953).
18. *Fortschr. Med.*, **8**, 7 (1890).
19. *J. Infectious Diseases*, **5**, 534 (1908).
20. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 197 (1901).
21. *J. Bacteriol.*, **5**, 527 (1920).
22. *J. Exptl. Med.*, **47**, 877 (1928).
23. *Brit. J. Exptl. Pathol.*, **5**, 271 (1924).
24. *Proc. Soc. Exptl. Biol. Med.*, **22**, 15 (1924).
25. *J. Dairy Sci.*, **14**, 276 (1931).
26. *Dairy Inds.*, **10**, 180 (1945).
27. *J. Dairy Research*, **17**, 253 (1950).
28. *J. Exptl. Med.*, **47**, 965 (1928).
29. *U. S. Public Health Mar. Hosp. Service Hyg. Lab. Bull.* 56, 457 (1909).
30. *J. Exptl. Med.*, **45**, 319 (1927).
31. *Vet. Record*, **62**, 27 (1950).
32. *J. Exptl. Med.*, **50**, 279 (1929).
33. *J. Exptl. Med.*, **51**, 327 (1930).
34. *J. Exptl. Med.*, **95**, 25 (1952).
35. *J. Exptl. Med.*, **95**, 39 (1952).
36. *J. Dairy Research*, **20**, 45 (1953).
37. *J. Infectious Diseases*, **48**, 408 (1931).
38. *Mich. Agr. Expt. Sta. Quart. Bull.*, **34**, 130 (1951).
39. *Intern. Assoc. Milk Sanitarians, 25th Ann. Rept.*, 105 (1936).
40. *Can. J. Technol.*, **29**, 232 (1951).
41. *Can. J. Technol.*, **29**, 238 (1951).
42. *Dairy Inds.*, **14**, 921 (1949).
43. *J. Milk and Food Technol.*, **15**, 181 (1952).
44. *J. Milk and Food Technol.*, **16**, 136 (1953).
45. *J. Milk and Food Technol.*, **13**, 353 (1950).

7 Milk Plant Processes

In the operation of dairy plants various processes are used that involve bacteriological considerations. These include (1) filtration and also clarification, to remove foreign material from milk, (2) centrifugal separation, to remove fat from milk, (3) homogenization, to divide the fat into smaller globules, and (4) pasteurization, to destroy a large percentage of the organisms in milk, cream, etc., including disease-producing species.

FILTRATION

Milk is filtered or strained on farms and also in dairy plants when clarifiers are not employed. The filtering materials used include cotton discs or webs and various types of cloth, some of which are manufactured especially for this purpose. In order to prevent excessive pressure at any point, the filtering material commonly is supported by a wire screen or other device. Cotton webs can be single or double faced to make them more resistant to damage during use. Special types of filters, intended to speed up the filtration of large volumes of milk, frequently are employed. Some have additional advantages; for example, the milk may be passed through the equipment in such a way that the sediment tends to fall away from the

filtering material and is not broken up and driven through or into it by pressure of the milk. Either gravity or a pump may be used to carry milk through a filter. Gathered cream intended for the manufacture of butter often is filtered through a special filter cloth or metal screen.

General Effect. The principal accomplishment of filtration of milk is the removal of a considerable portion of the visible dirt. Ordinarily, this is regarded as of sufficient importance to justify the practice. It would be ideal if all milk could be produced under such conditions that there would be no dirt in it, but even with excellent methods some sediment is present and it seems logical to remove as much of it as possible. Filtration should not be used to conceal unsatisfactory methods of milk production, and excessive amounts of sediment in milk justify its rejection.

Various factors influence the rate and efficiency of milk filtration. In addition to the type of filter and filtering material, the following properties of the milk are important: Content of extraneous matter, presence of certain products of udder infections, temperature, and composition, especially fat content.

Effect on Bacterial Content. In general, filtration of milk has only a negligible effect on the bacterial content. Although some of the organisms in the foreign material are distributed through the milk, others are not. The pouring or pumping of milk against a filter tends to break up some of the sediment and thus set free more organisms than if the particles had remained intact.

Stocking [1] strained milk through 2 thicknesses of cheesecloth, supported by a wire gauze, immediately after it was drawn. In 7 trials the dirt removed by straining varied from 10.8 to 91.8% and averaged 46.6. The plate counts on unstrained and strained milk, respectively, were 11,400 and 10,150 per ml. The period required to curdle at 70 F was the same with unstrained and strained milk in 4 comparisons; strained samples curdled more quickly in 3 comparisons. Conn and Stocking [2] made 10 comparisons of plate counts on unstrained and strained milk; the milk was drawn from individual cows into an ordinary pail and, after removal of a sample, was strained through 2 layers of sterilized cheesecloth. The average counts on unstrained and strained milk, respectively, were 13,743 and 13,005 per ml. The period required to curdle at 70 F varied greatly, but on the average the unstrained samples curdled 4 hr earlier than the strained.

Effect of filtering milk through 3 separate layers of absorbent cotton

in a special apparatus used on a farm producing milk of high sanitary quality was studied by Heinemann *et al.*[3] Samples of milk were taken from the can, from the top of the filter, and after filtering. Five sets of samples were obtained each day for 16 days. The average plate counts per milliliter were: Before filtering 2,060; on top of filter 3,912; and after filtering 2,790. The investigators suggested that the higher counts on top of the filter and after filtering may have been due to the breaking up of clumps of bacteria by the agitation.

Filtering of milk in an apparatus in which the flow was upward through a filter cloth was studied by Lucas *et al.*[4] With 68 lots of milk filtered at 85 to 90 F, the effects on the plate counts were: 18 (26%) of the lots showed increases of 3 to 81% (average 24.4), 44 (65%) showed decreases of 2 to 53% (average 19.3), and 6 (9%) showed no change. With 74 lots filtered at 55 to 70 F, the following changes in the counts occurred: 20 (27%) of the lots showed increases of 4 to 114% (average 24.7), 40 (54%) showed decreases of 5 to 88% (average 29.3), and 14 (19%) showed no change. The filter tended to lower rather than increase the plate count, but a reduction was by no means regularly obtained. The filter had little selective action in removing bacteria, although peptonizers seemed to pass through more readily than other groups. Filtration had no appreciable effect on keeping quality or cream line of the milk. It was very effective in removing visible dirt, being almost as efficient as clarification.

Jacobsen and Olson[5] noted that filtering milk through a special type filter had only a slight effect on the bacterial content, as determined by either the plate method or microscopic count. When carried out at 60 F filtration had no appreciable effect on the cream line but at 90 or 110 F it was decreased slightly.

Filtering milk through a bed of modified starch removed many bacteria from it in the trials of Inglesent and Storrow.[6] They filtered some lots of milk through filter cloth coated with 1 oz of modified starch (starch partly gelatinized by roll-drying of an aqueous suspension) per sq ft of filtering surface, and obtained the bacterial counts given on p. 192.

The authors noted that the modified starch should be free of soluble starch and care should be exercised in its selection since some lots contain many bacteria. The modified starch on the filter cloth reduced the size of many of the fat globules, as shown by microscopic examination, but it did not reduce the fat content of the milk.

Bacteria per Milliliter before and after Milk Was Filtered

Test No.	Regular Filter Total Count		Regular Filter Coated with Modified Starch Total Count	
	Before Filtration	After Filtration	Before Filtration	After Filtration
1	210,000	200,000	150,000	50,000
2	360,000	330,000	209,000	47,000
3	170,000	165,000	120,000	60,000
4	15,000	14,000	243,000	41,500
5	270,000	253,000	144,000	30,000
6	310,000	300,000	250,000	21,500
7	21,000	23,500	401,000	10,700
8			290,000	110,000
9			301,000	10,000

Contamination from Filters. In considering the effect of filtration on the bacterial content of milk, the possibility of the equipment, especially a previously used filter cloth, being the source of large numbers of bacteria must be recognized. When a cloth is carelessly washed without any attempt at sterilization or when it is left moist for a considerable period so that growth of organisms occurs, heavy contamination of the milk during the next use is to be expected.

A filter cloth that is to be used again should be thoroughly washed, then boiled or steamed, and quickly dried if it is to be kept in a suitable condition. Such treatment tends to reduce the efficiency. Lucas *et al.*[4] found that a filter cloth used once and sterilized was still fairly efficient but, for dirty milk, the efficiency was reduced to approximately 80% by a second washing; they noted that with fairly clean milk a cloth can be used successfully longer than with dirty milk. In many plants employing filters, a cloth is used only once.

In two milk plants, one using a gravity filter and one a pressure filter, Dahlberg and Marquardt[7] found that accumulation of sediment on the filter cloth did not affect the efficiency; they pointed out that this suggests the sediment remained on the cloth and was not washed into the milk. Leucocytes were not removed by filtration.

The various materials used to filter milk ordinarily come from the manufacturer in a satisfactory condition bacteriologically, but they may be contaminated by exposure. They also may be contaminated by careless handling; such contamination is common with the cotton discs often used to filter milk on farms.

CLARIFICATION

Clarification of milk had its beginning in the employment of a separator to throw out foreign material gaining entrance during production and handling. There were certain objections to the use of a separator for this purpose, such as the necessity of mixing the skim milk and cream and occasional interference with the creaming ability of the remixed milk and, accordingly, machines were designed for removal of sediment instead of separation of the fat.

Composition of Slime. The material collecting in the bowl of a clarifier is made up of dirt of various kinds that has fallen into the milk, body cells, bacteria, particles of casein, fat, and other materials. Erythrocytes are present occasionally, even when appearance of the milk does not suggest that it contains blood, and these may be deposited as a very definite layer in the bowl.

With 8 samples of clarifier slime, McInerney [8] found the average composition to be water 71.33%, total solids 28.67%, fat 4.0%, ash 3.15%, and casein 2.13%. Jacobsen and Olson [5] reported that, on a moisture-free basis, the material removed by clarification averaged about 60% protein, 8% fat, and 10% ash; with various clarification temperatures the amounts of protein and fat in the slime were greatest at the lowest temperature, but the amount of ash was rather constant.

The numbers of bacteria and body cells in slime vary widely, presumably because of variations in the numbers in the milk and also in the amounts of other constituents in the slime. Bacterial counts have been reported [9,10,11] from 17,000 to 20 billion per g, with the lower values tending to represent milk low in count. Body cell counts reported [9,10,11] range from 3 million to 1,295 million per g; both low and comparatively high counts have been obtained on slime from certified milk.

Amount of Slime. The amount of clarifier slime obtained from milk is extremely variable and depends, to a large extent, on the amount of dirt and number of body cells in the milk. Other factors sometimes are involved; for example, development of a small amount of acid in milk may so influence the casein that considerable quantities are thrown into the slime. In all cases, however, the amount of slime is small compared to the volume of milk clarified. Cows vary greatly in the amount of slime in the milk under apparently normal conditions, and with individual animals there often is a variation from one milking to the next. The amount of slime is increased by ab-

normalities of the udder. Cows that have just freshened give a high percentage of slime which often continues for several weeks.[11] Jacobsen and Olson[7] reported the dry weights of slime removed per 1,000 lb of milk were 39.08 g in 8 trials at 60 F, 28.02 g in 10 trials at 90 F, and 24.14 g in 6 trials at 110 F.

Figure 24. Material collected in clarifier bowl.

Effect on Bacterial Content. The bacterial content of clarifier slime shows that organisms regularly are removed from milk by clarification. However, the number of bacteria, as determined by the plate method, often is greater in clarified milk than in unclarified milk from the same lot. The increase is due to breaking up of clumps of bacteria during the agitation in the machine so that a clump which would have given only 1 colony before clarification gives >1 after clarification; accordingly, the increase is an *apparent* one. Removal of bacteria and breaking up of clumps are the two factors tending to change the plate count on milk during clarification, and whether there will be a de-

crease or an apparent increase is determined by which of these factors has the greater influence.

Early studies [9,10,11] showed that clarification causes both decreases and apparent increases in the plate count on milk, with increases being the more common. Both the decreases and increases show great variation, percentage-wise. With some lots of milk, the increase is surprisingly large.

More recent trials confirm the early results. Lucas *et al.*[1] studied the effect of clarification on the bacterial counts of 49 samples of milk, using temperatures ranging from 85 to 92 F; after clarification 35 (71%) showed increases which averaged 28.1% (from 1 to 100), 9 (19%) showed decreases which averaged 11.7% (from 2 to 23), and 5 (10%) showed no change. Clarification at 55 to 65 F influenced the bacterial content of 97 samples as follows: With 60 (62%) there were increases which averaged 25.9% (from 2 to 220), with 23 (24%) there were decreases which averaged 12.2% (from 3 to 26), and with 14 (14%) there was no change.

Both increases and decreases in the plate count on milk as a result of clarification were noted by Wilster; [12] considering the 43 comparisons, there was an average increase of 12.1%. When the rate of inflow of milk was reduced, clarification always caused a decrease in the plate count, the average reduction for 19 trials being 55.3%. With either a normal or reduced inflow, clarification had no significant effect on the types of bacteria present or on the fermentation that occurred at a temperature favorable for bacterial growth.

Jacobsen and Olson [5] found that clarification at all temperatures studied increased the plate count of milk. The increases averaged 134.5% at 60 F, 36.1% at 90 F, and 20.0% at 110 F. Microscopic examinations indicated that the increases were due to breaking up of clumps. Weinreich [13] reported on work done by Parfitt in which large bacterial reductions were obtained when milk was clarified hot (about 140 F), but increases in counts were obtained when the milk was clarified cold (about 66 F).

Effect on Body Cell Content. Because body cells in milk are counted directly and there is no opportunity for an *apparent* change in number, the presence of body cells in clarifier slime indicates that clarified milk has a lower cell count than the same milk before clarification. In the early trials, 52 samples of milk [9] gave decreases in cell counts from 7 to 73% (average 39), 63 samples [10] gave decreases from 11 to 78% (average 41), 25 samples of certified milk [11] gave decreases

from 46 to 92%, and 6 samples of market milk [11] gave decreases from 74 to 92%.

Jacobsen and Olson [5] noted that the temperature of clarification influenced the percentages of cells removed. At 60 F, 34.98% were removed; at 90 F, 46.28%; and at 110 F, 53.20%. Hahn and Tracy [14] found that single clarification, using either a clarifier unit or a separator unit and remixing the milk, reduced the cell content on an average of 61.7 and 51%, respectively. The data indicated variations in the numbers of cells removed from milk throughout the entire clarifying period. Efficiency of cell removal increased with an increase in temperature. Clarifying two or more times increased the extent of cell removal over single clarification. Pumping milk at slow speeds through an air-tight clarifier resulted in greater clarifier efficiency than pumping at higher speeds. Clarification after homogenization was more efficient than before homogenization. In comparing clarification at 40 and 135 F, Demott *et al.*[15] found no difference statistically in cell removal.

Removal of Visible Dirt. Clarification very efficiently removes the visible dirt from milk. Removal of 99%,[8] and more,[4] has been reported. At various temperatures a clarifier removes any particles large enough to be caught on a filter pad.[15] Some of the dirt which gets into milk either goes into solution or is so finely divided it ordinarily is not visible and, accordingly, a portion of the dirt may not be removed by clarification; different types of dirt vary greatly in this respect.

Effect on Keeping Quality of Milk. Clarification has no significant effect on the keeping quality of milk. In clarified milk, acid increase,[10] bacterial growth,[8] and reduction of methylene blue [12,16] may be more rapid or less rapid than before clarification. Slight reduction in the keeping quality of clarified milk has been attributed to stimulation of the acid-producing bacteria.[4]

Effect of Full Clarifier Bowl. Removal of bacteria, body cells, and sediment by clarification can only take place when there is room for them in the bowl [13] and, if a clarifier is to be used for an extended period, it may be necessary to stop the operation and clean the bowl.

Effect on Pathogens. There is no evidence that clarification makes milk safe by removal of pathogens. Traum and Hart [17] inoculated 14 samples of clarified milk from a tuberculous herd into guinea pigs; 11 samples caused tuberculosis in all the animals inoculated, 2 produced tuberculosis in one guinea pig while the other died soon after inoculation, and 1 caused death of both guinea pigs through septicemia.

Value. The primary value of clarification is the removal of foreign material from milk. Another advantage is the elimination of a portion of the body cells from milk; these cells cannot be considered harmful, but their removal is advisable from the aesthetic standpoint.

CENTRIFUGAL SEPARATION

In centrifugal separation, milk is divided into three fractions, cream, skim milk, and slime. Each fraction contains bacteria. Plate counts on all three fractions are influenced by the breaking up of clumps of bacteria during the centrifuging. The numbers of bacteria in cream compared to the numbers in the original milk are of special importance from such standpoints as bacterial standards for cream and bacteriology of products made with cream.

Distribution of Bacteria During Separation. Early studies on centrifugal separation indicated that the cream frequently contains somewhat larger numbers of bacteria per milliliter and the skim milk somewhat smaller numbers than the original whole milk. In a typical trial Eckles and Barnes [18] found that the whole milk contained 58,050 bacteria per ml, the skim milk 16,200, the cream 98,000, and the slime approximately 38,000,000. Average counts obtained by Anderson [19] in 7 trials were: Whole milk 619,800 per ml, skim milk 119,700, and cream 4,500,000.

The numbers of bacteria in cream are influenced by the tendency of bacteria to become entangled in the clumps of fat globules during separation and be carried with them. In some trials the cream contains smaller numbers of bacteria per milliliter than the skim milk, and it appears that various factors influence the bacterial distribution. Lamson [20] noted that there usually were less, or only slightly more, bacteria per milliliter in separator cream than in the whole milk from which it came, and the skim milk usually contained fewer bacteria than the whole milk.

Leete [21] concluded that centrifugal separation with a clean separator will not result in cream having a greatly higher count than the whole milk from which it is obtained. In 100 runs with a steam-turbine separator the average plate counts per milliliter were: Whole milk 435,240, skim milk 312,740, and cream 500,830. Seventy samples of the cream showed increases in count over the corresponding whole milk, with an average of 23.87%; 23 samples showed decreases, with an average of 14.06%; with 7 samples the counts on the cream and

whole milk were the same. Considering the 100 samples, Leete found the average increase of the cream over the whole milk was 14.84%. The fat content of the whole milk averaged 3.9% and of the cream 34.6%. Both the greatest increases and greatest decreases in the bacterial content of the cream over the whole milk occurred with low-count milk.

Separator Slime. The slime that collects in a separator bowl contains essentially the same materials as clarifier slime, including bacteria and body cells. Dunn *et al.*[22] found from 700 to 900 million body cells per g. In general, a separator is very efficient in removing foreign material from milk.

Effect on Pathogens. Separation does not have a selective action on the organisms in milk, and there is no complete concentration of the pathogens in either the slime or cream. Moore [23] studied the effect of centrifugal separation on *M. tuberculosis* in milk and found that, although many of the organisms were thrown into the slime, both the skim milk and cream contained them.

HOMOGENIZATION

Homogenizers are used extensively in the preparation of homogenized market milk, market cream, frozen dessert mixes, etc. Construction of the early models made them difficult to keep in a satisfactory bacteriological condition, but construction changes,[24] prompted in part by sanitary considerations, have simplified the care of the modern machines. However, the pressures under which the machines sometimes operate force solids under gaskets, valves, etc., so that adequate care still is a problem.

Breaking up of Clumps of Bacteria. Study of the contamination from a homogenizer is complicated by the breaking up of clumps of organisms in material run through it, so that an increase in the plate count may not be due entirely to addition of organisms. In some instances contamination is very clearly shown by the presence of species in the homogenized material that are lacking in the unhomogenized product. Plating sterile water run through a machine may give a more accurate idea of the contamination than plating a product before and after homogenization.

James [25] found that the influence of the breaking up of clumps of organisms during homogenization on the plate count of cream was fairly constant during the handling of one lot, but that there was

considerable variation between lots. Little difference was noted between pressures of 750 and 2,500 lb per sq in., which suggests that the lower pressure was sufficient to break up the clumps. Five runs were made with pasteurized skim milk, using a machine that was practically sterile, and the counts remained fairly constant during one operation; rehomogenization had little influence on the count so that homogenizing once evidently was as effective in breaking up clumps as homogenizing twice. Comparative counts before and after homogenization varied from a ratio of 1 to 1.5 to one of 1 to 4.6.

In trials by Corbett,[26] the plate count on sterile milk inoculated with *M. pyogenes* var. *aureus* (showing many clumps) was greatly increased by the breaking up of clumps during homogenization, whereas the count on milk inoculated with *E. coli* (largely as single cells) was not significantly changed.

Contamination from Homogenizers. Contamination from a homogenizer varies greatly, depending largely on the care given the machine. Apparently, clumps of bacteria picked up from a machine also can be broken up during the homogenization.[26]

The piston packing has been stressed as a source of bacteria in a homogenizer. Bortree and Lucas[27] found that elimination of contamination with *E. coli* is extremely difficult without special precautions in cleaning and sanitizing every part of the homogenizer with which the milk comes in contact. They noted that one of the focal points of contamination is the leather gaskets. By introducing another set, thus permitting every other day use, which allowed one set to be soaked longer in the chlorine solution and yet be adequately dried, the difficulty with *E. coli* contamination was overcome. A saturated salt solution also has been suggested for holding the packing.[26] Adequate care in the sanitizing of a homogenizer is essential if contamination from it is to be avoided.

Counts on Homogenized Milk. Although breaking up of clumps of bacteria and contamination from the homogenizer tend to increase the plate count of homogenized milk, somewhat higher pasteurization exposures commonly are used with it since creaming ability is not involved, and this acts as a compensating factor.

Plate counts on commercial homogenized milk are more or less comparable with those on unhomogenized milk and readily are kept within normal standards. However, counts tend to be slightly higher on the homogenized product. Corbett[26] reported monthly averages on regular and homogenized milk, taken from the same vat, as follows:

Monthly Averages of Plate Counts on Regular and Homogenized Milk Taken from the Same Vat

Month	Bacteria per Milliliter	
	Regular Milk	Homogenized Milk
Aug.	690	752
Sept.	472	1,248
Oct.	418	1,783
Nov.	284	2,289
Dec.	289	1,108
Jan.	328	871
Feb.	248	968
Mar.	152	968
Apr.	133	1,501
May	171	1,845
June	414	2,322
July	427	2,202

The following data [26] again show the tendency of homogenized milk to have a higher plate count than the unhomogenized milk.

Plate Counts on Unhomogenized and Homogenized Milk. Milk Homogenized after Pasteurization, Using a New Sanitary-type Machine

	Bacteria per Milliliter
Milk from vat	1,210
First milk through homogenizer, no pressure	1,980
Unhomogenized milk, beginning of run	1,182
Homogenized milk, beginning of run	1,990
Unhomogenized milk, middle of run	1,158
Homogenized milk, middle of run	1,510
Unhomogenized milk, end of run	1,180
Homogenized milk, end of run	1,418

Pasteurization of Homogenized Milk. Homogenized market milk always is pasteurized because in raw homogenized milk the normal milk lipase may act very rapidly on the small fat globules and the product quickly become rancid. Milk has been pasteurized both before and after homogenization, but from the bacteriological standpoint pasteurization following homogenization is preferable since it tends to control contamination from the homogenizer. Doan [28] suggested that clarification following homogenization may be more efficient in throwing out bacterial cells and thus compensate for the effect of the homogenizer.

Some plants have had homogenized market milk become rancid very quickly. When this occurs it is advisable to test the product with the phosphatase test to make certain that a leaky valve, or some other irregularity, has not added raw milk to it. If homogenized milk is added to raw milk, development of rancidity sometimes is extremely rapid. Such an addition may occur if diversion of the milk becomes necessary, because of some cause such as a delay in the filling operation, and it is diverted to the raw milk holding tank.

Keeping Quality of Homogenized Milk. In a trial carried out by Corbett,[26] growth of bacteria in both homogenized and unhomogenized milk was well controlled for 48 hr at 56 F, but at 70 F growth in the homogenized milk was much more rapid than in the unhomogenized product. This suggests that organisms added to milk by a homogenizer, even when the numbers are small, may have a significant effect on the spoilage at high holding temperatures. With adequate cleaning and sterilization of a homogenizer, psychrophiles would not be expected in it.

PASTEURIZATION

Milk, cream, etc., commonly are heated to destroy many of the organisms in them. The bacteria of special significance in this connection are disease-producing species, but types that bring about spoilage of dairy products also are of importance. When the heating meets certain requirements, the process is referred to as pasteurization.

In the establishment of acceptable exposures to heat, two factors are involved, temperature and holding period. A temperature which is ineffective if used for a short period may be entirely satisfactory when the period is prolonged. Cooling immediately following the heating, to prevent growth of the organisms surviving, normally is regarded as a part of the process; however, when milk or cream is to be used for products involving growth of organisms, cooling need only be to the temperature employed for such development. Milk regulations generally include requirements with reference to minimum pasturization exposures (temperature and time). These commonly are exceeded with milk and cream for various manufacturing purposes.

Origin. The word *pasteurization* is derived from the name of Louis Pasteur, a French scientist who developed the process. Pasteur wished to prevent abnormalities in wine, such as formation of acid, ropiness, etc., and found (1860 to 1864) that by heating wine for a few minutes to 122 to 140 F the undesirable changes could be prevented. In 1868,

heated wine was sent around the world successfully on a French frigate. Later, Pasteur found that heating also prevented undesirable changes in beer. Pasteur was not the first to use heat to control fermentations. As early as 1782, Scheele heated vinegar to prevent spoilage. In 1810, Appert preserved various foods, including milk and cream, by application of heat to closed containers. Apparently, the heating of milk and cream for certain manufacturing purposes was practiced before the time of Pasteur. However, the studies of Pasteur are outstanding in that they were based on a knowledge of the role of organisms in causing the changes to be controlled.

In 1886, Soxhlet advised the heating of milk for infant feeding. The milk needed for a day was divided and that required for each feeding was put into a bottle of a type that could be cleaned and sterilized easily, after which the bottles were heated in hot water. Soxhlet incorrectly believed that milk treated according to this method was sterile. Heating milk for general consumption and cream for butter manufacture followed rapidly, and pasteurization soon was well established in the dairy industry.

Types. With dairy products two main types of pasteurization are employed: (1) The low-temperature, long-time (LTLT) or holding method, and (2) the high-temperature, short-time (HTST) method which, before adequate control of the temperature and period of heating had been perfected, often was referred to as continuous or flash pasteurization.

With the LTLT method of pasteurization an exposure to at least 143 F for at least 30 min usually is required. The process commonly is carried out in vats or holding compartments. For a large operation a series of holding compartments can be employed so that one compartment always is supplying milk to the bottling equipment; they may be filled and emptied automatically. Pasteurization of milk and cream in closed bottles, which is referred to as final package pasteurization, is essentially a LTLT method, its chief advantage being elimination of contamination following the heating. However, with it, heating and cooling through the bottle wall are slow and there may be variations in the temperature of the product in a bottle.

With the HTST method of pasteurization an exposure to at least 161 F for at least 15 sec commonly is required. The short holding period permits the construction of equipment to which, and from which, the milk flows in a continuous stream. Plate, tube, and electric heaters have been developed. With milk or cream for certain purposes, heating is accomplished by the direct introduction of steam.

Such exposure also is combined with vacuum treatment in special equipment which removes certain off flavors.

At one time regulatory officials did not accept short time pasteurization for market milk because of irregularities in the heating and the tendency to use inadequate exposures in an attempt to avoid overheating portions of the milk. Now, it generally is accepted because equipment has been so improved that heating of the milk is uniform and underheating of a portion is avoided by flow diversion valves.

Regenerative systems, in which the milk to be pasteurized is heated partially and the pasteurized milk cooled partially through an exchange of heat between them, are useful when large volumes of milk are being treated with either process. HTST pasteurization rather regularly makes use of such a system.

Basis for Present Pasteurization Exposures. Two effects of the heat applied to milk must be taken into account whenever a pasteurization exposure is under consideration: (1) Bacterial efficiency, and (2) influence on chemical and physical properties. If bacterial efficiency were the only consideration, relatively high exposures might be advisable but the other effects may require that the exposures be reasonably low. In general, exposures are selected for market milk that ensure destruction of the pathogens present and have as little influence as possible on flavor and cream line. A slight heated flavor in market milk is not objectionable to many people and is preferred by some; also, it tends to mask certain slight off flavors, such as feed and absorbed flavors. However, with good raw-milk supplies, the tendency is to give the milk just as little heated flavor as possible through adequate control of the heat treatment. Market cream frequently is heated to higher exposures than those used with milk. With milk intended for cheese, excessive pasteurization may interfere with its coagulability. In the case of cream to be churned, high pasteurization may have a desirable effect on fat stability.

Bacterial Efficiency. To be satisfactory from the standpoint of bacterial efficiency, pasteurization must kill a reasonable percentage of the total bacteria present, including the pathogens. Bacterial efficiency can be expressed as the percentage of the total bacteria that are killed by the heat, but this is not satisfactory because, with excessive numbers of bacteria present originally, a high percentage may be destroyed by exposures which still leave many organisms and do not kill all pathogens. In general, with a given exposure, the higher the initial count the greater will be the percentage of organisms killed. Requirements dealing with destruction of bacteria during pasteuriza-

tion ordinarily refer to the numbers permitted both before and after the heating.

Destruction of pathogens that may be in milk is the important point to consider with reference to the efficiencies of pasteurization exposures. Present exposures are based primarily on the heat resistance of *M. tuberculosis* since it has been considered relatively resistant for milk-borne pathogens.

Heat Resistance of Pathogens

M. tuberculosis. Early investigations on the exposure to heat required to destroy *M. tuberculosis* in milk yielded variable results,[29] with the findings of one investigator often showing pronounced variations. In 1899, Smith [30] explained this by pointing out that formation of a scum or pellicle on the milk, due to evaporation, enabled the organism to survive when otherwise it would have been killed. He found that, when suspended in milk, *M. tuberculosis* was killed at 140 F in 15 min if no pellicle was allowed to form, but with a pellicle it might survive for 60 min. Russell and Hastings [31] reported that heating milk in a tightly closed commercial pasteurizer at 140 F for 10 min destroyed *M. tuberculosis* in it; they recommended an exposure of 140 F for not less than 20 min in order to ensure pasteurization. Rosenau [32] found that the organism in milk was killed when heated at 140 F for 20 min or at 149 F for a much shorter period.

Studies of Traum and Hart [33] are particularly significant since they involved milk from a heavily infected tuberculous herd that was intended for consumption. The diseased animals, which at one time numbered 500, were kept separate, and the milk from them was pasteurized and sold. Pasteurization was carried out in three well-insulated vats. Steam was turned into the circulating water as soon as milk began to flow into a vat and 20 to 30 min were required to fill the vat and bring the milk to the pasteurizing temperature; emptying the vat required 15 or 20 min, so that the last milk leaving the vat received considerable additional exposure before running over the cooler. Temperatures were obtained with a recording thermometer, but part of the time a thermometer also was placed directly in the milk. The milk was examined at a distant point, within 24 to 48 hr of collection, by centrifuging 90 to 95 ml and inoculating the cream and sediment subcutaneously or intramuscularly into 2 or 3 guinea pigs; reinoculations were made in all cases of suspicious lesions. Of the 24 samples of raw milk studied, all but 1 produced tuberculosis in inoculated animals and with it the guinea pigs died of septicemia.

After pasteurization, 23 samples failed to produce tuberculosis or cause death in a single instance; 12 of the samples were heated above 140 F for longer than 20 min, whereas the remaining 11 were heated to 140 F and held for only 20 min.

Park [34] reported that the following exposures were required to kill *M. tuberculosis* in milk:

132 F	60 min	140 F	15 min	155 F	1 min
134 F	40 min	142 F	10 min	160 F	30 sec
136 F	30 min	145 F	6 min	170 F	20 sec
138 F	20 min	150 F	2 min		

He also noted that, of 100 samples of ordinary milk, 20% contained the organism before pasteurization and none after pasteurization. In tests by Minett and Pullinger [35] every one of 43 samples of milk coming into London in 3,000-gal tanks contained *M. tuberculosis,* whereas the same number of samples after pasteurization were negative. Price [36] found in Canada that 26% of 100 samples of mixed raw milk contained *M. tuberculosis,* and all of the 100 samples examined immediately after pasteurization were negative.

Other studies confirm the general results on the heat resistance of *M. tuberculosis.*[37–41] Occasionally, exposures commonly considered to destroy the organism have appeared not to do so,[42,43] but these irregularities have not prevented the establishment of the exposures which are accepted as killing the organism.

Oldenbusch *et al.*[44] found that *M. tuberculosis* (bovine) was killed in cream in <20 min at 135 F, in <10 min at 140 F, in <7 min at 142 to 143.5 F, and in <3 min at 145 F. In studies by Bundesen *et al.*[45] the organism failed to infect guinea pigs after exposure to test temperatures from 160 to 180 F for periods of 15 to 30 sec in chocolate milk, 12% milk, and 12% ice cream mix.

***Brucella* Organisms.** Rosenau [32] noted that *Br. melitensis* was not destroyed at 131 F for a short time but was killed at 140 F, and the majority of the organisms were dead at 136.4 F. Evans [46] reported that 125 F for 30 min or 145 F for 30 sec killed *Br. abortus.* Carpenter and Boak [47] noted that human, porcine, and bovine cultures of *Brucella* were not viable after an exposure to 140 F for 20 min. Park [48] found that when heated in milk *Br. melitensis* and *Br. abortus* survived 140 F for 7.5 min but were killed in 10 min; at 142 F they survived 5 min but were killed in 7.5 min; at 145 F they were killed in 5 min. Carpenter and Boak [49] reported that 20.4% of 122 samples of raw milk from 67 villages and 2 cities showed evidence of *Br. abortus* infection

when 2 ml of cream from each sample was injected subcutaneously into guinea pigs, whereas all of 205 samples of pasteurized milk and cream from 12 villages and 38 cities were negative. In London,[35] 27 of 43 samples of raw milk from 3,000-gal tanks contained *Br. abortus*, and the same number of samples of pasteurized milk were negative. Murray *et al.*[50] found that a temperature of 143.6 to 145.4 F for 3 min was sufficient to destroy *Brucella* organisms, both bovine and porcine. Additional studies [51] also show that the *Brucella* organisms are more easily destroyed by heat than *M. tuberculosis*.

Additional Pathogens. Rosenau [32] found that *S. typhosa* was killed in milk heated to 140 F and maintained there for 2 min; the great majority of the organisms was killed by the time the milk reached 138.2 F, and only a few survived at 140 F. *Corynebacterium diphtheriae* often failed to grow after the milk reached 131 F, although occasionally it survived until the milk reached 140 F. The resistance of *Vibrio comma* was similar to that of *C. diphtheriae*. The dysentery organisms (Shiga and Flexner) withstood 140 F for 5 min but were killed at this temperature in 10 min. Rosenow and Hess [52] found that an organism responsible for an outbreak of septic sore throat was destroyed at 140 F in 20 min.

Krumwiede and Noble [53] investigated the heat resistance in milk of 27 *S. typhosa* cultures recently isolated from carriers, 7 *Salmonella paratyphi* cultures, 12 *Salmonella schottmuelleri* cultures, and 4 *Salmonella enteritidis* cultures. The milk was sterilized, cooled, and tested for sterility. It then was heated to 140 F in a water bath, suspensions of the organisms in salt solution added, and rubber stoppers inserted. After shaking, it was returned to the water bath and held at 140 F for 15 min. Following rapid cooling, the milk was held 48 hr at 98.6 F to allow multiplication of any organisms that survived. None of the cultures yielded growth.

In trials carried out with commercial pasteurizers,[54] *S. typhosa* was destroyed in one series of tests by momentary heating to 141 F and by one type of holder at 132 F for 30 min; *C. diphtheriae* and hemolytic streptococci were destroyed by momentary heating to 129 F. Park [48] reported that 200 strains of hemolytic streptococci obtained from cases of septic sore throat, scarlet fever, erysipelas, and other diseases known to be due to hemolytic streptococci were all killed at 140 F for 30 min when heated in milk. The majority of them was killed at 136 F for 30 min. Oldenbusch *et al.*[44] found that 2 strains of streptococci, 1 from a case of scarlet fever and 1 from a septic sore throat, did not survive in cream at 135 F for 5 min, or at 140 to 142 F

for 1 min. *S. typhosa* was killed at 135 F in <10 min, at 140 F in <5 min, and at 142 to 145 F in <3 min. In ice cream mix the streptococci and *S. typhosa* were killed in <5 min at 145 to 150 F, and *C. diphtheriae* did not survive these temperatures for 0.5 min.

Poliomyelitis Virus. Kaplan and Melnick [55] reported that strains of poliomyelitis virus from human feces and spinal cords of infected monkeys, when suspended in water, milk, or ice cream, were destroyed by laboratory pasteurization at 143 F for 30 min, or 160 F for 15 sec. Milk and ice cream exerted a protective effect on the virus, as compared with water, but, since this effect was manifest at temperatures of 131 F, or lower, it was of little practical significance. A rodent-adapted strain of poliomyelitis virus suspended in cream survived 160 F for periods up to 2 min. The Lansing strain of the virus suspended in cream was inactivated by an exposure to 160 F for 45 sec.

Q Fever Organism. The Q fever organism (*Coxiella burnetii*) apparently sometimes survives pasteurization. Huebner *et al.*[56] carried out tests on its heat resistance in 5 plants. The serum reactions shown by guinea pigs and mice in the complement fixation test for Q fever served as the test for the presence or absence of infection in the milk. The original raw milk used in the tests was highly infectious since 92.3% of the guinea pigs injected with it reacted positively. All the 36 guinea pigs injected with HTST pasteurized milk (160.5 F for 15 sec) reacted negatively while 3 out of 48 guinea pigs injected with LTLT pasteurized milk (143 F for 30 min) reacted positively. Three out of 32 samples taken from bottles of LTLT pasteurized milk and 1 out of 4 samples of LTLT pasteurized cream were positive.

Pasteurization of milk naturally infected with *C. burnetii* was carried out at 11 plants using LTLT pasteurization (143 F for 30 min) by Lennette *et al.*[57] The organism survived in 1 of 35 trials, as judged by the presence of complement-fixing antibody titers to *C. burnetii* in the sera of inoculated guinea pigs. In similar tests at 6 plants using HTST pasteurization (160 F for 15 sec), 2 out of 42 milk samples reacted positively.

Enright *et al.*[58] noted that since, in the opinion of some investigators, dead *C. burnetii* may be immunogenic, it becomes necessary to make at least one subpassage in guinea pigs to ascertain the viability of the organisms in the inoculum. Certain laboratory strains of the organism have been adapted to growth in the yolk-sac of developing chick embryos, and this provides another method of demonstrating viability. The investigators found that when a milk sample contains 10,000 infectious guinea pig doses of *C. burnetii* per ml, the maximum thus far

demonstrated in the milk of infected cows, enough organisms may survive 143 F for 30 min to infect guinea pigs and embryonating eggs. They considered current information inadequate to allow evaluation of the efficiency of HTST pasteurization in eliminating *C. burnetii* from milk.

Additional Considerations

Margin of Safety in Pasteurization. Since under plant conditions temperature and time of heating may not be controlled with great accuracy, pasteurization regulations include a margin of safety over the exposures required to kill *M. tuberculosis.* This compensates for minor irregularities in the heating.

Influence of Pellicle Formation on Heat Resistance of Organisms. The protection given *M. tuberculosis* by the pellicle formed on heated milk undoubtedly also occurs with other organisms. Russell and Hastings [59] inoculated milk with a coccus and found that the pellicle which developed on heating yielded growth when the milk below did not. Removal of a pellicle resulted in formation of another, but this was sterile. When a pellicle containing organisms was heated in water, the organisms resisted temperatures that ordinarily killed them.

Increased resistance of organisms in the pellicle developed on heated milk evidently is due to more than one factor. The pellicle forms as a result of evaporation, and this requires heat so that the temperature undoubtedly is lowered even if no decrease can be detected by ordinary measurements. It is probable also that the pellicle offers some protection, either by its resistance to heat penetration or in some other way. The influence of pellicle formation on survival of organisms during pasteurization is of importance under practical conditions, and pellicle development is prevented, either by agitation or by heating and holding under conditions which limit evaporation.

Importance of Uniform Heating. Reasonable uniformity in both temperature and time of heating for all portions of a lot of milk or cream is highly desirable. It is essential that all the material be heated sufficiently, whereas overheating portions may have undesirable effects.

Uniform heating of a large volume of milk presents a difficult engineering problem. Some of the common causes of underheating a portion of a lot are (1) leaky inlet or outlet valves which permit some of the material to escape the desired heat exposure, (2) dead ends which retain material in such a way that it does not mix properly with that coming in contact with the heating surface, (3) splashing that carries material to parts of the equipment where it does not receive sufficient

heating, (4) formation of foam through which heat is transferred only slowly, and (5) ineffective flow diversion valves. Overheating may result from (1) a high temperature heating surface which supplies heat so rapidly that the material next to it is heated significantly above the temperature desired for the entire lot, and (2) unnecessarily long holding, such as occurs when the equipment heats or cools so slowly that the material is near the pasteurization temperature for extended periods. Dairy engineers have made much progress in designing equipment that corrects or limits these irregularities. This has brought flush valves, nonleak valves, foam heaters, and other refinements. Well-designed plate-type heaters and coolers are of special importance in this connection.

Foam in LTLT Pasteurization. Formation of foam on milk during LTLT pasteurization is of considerable bacteriological significance. Whittaker *et al.*[60] studied the temperatures of milk and foam during commercial pasteurization by means of thermocouples and found, in 19 runs, that the temperature of the foam always was lower than that of the corresponding milk. The average temperature of the foam was 5 F, or more, below that of the milk in 84.2% of the tests at the beginning of the holding, in 73.3% at the middle of the holding, and in 84.6% at the end of the holding. Temperature of the foam frequently was more than 11 F below that of the milk and in one instance was 18.1 F below. The foam and corresponding milk were examined for numbers of bacteria, and at both beginning and end of the holding the foam showed the higher count in every instance. Although the milk gave the expected decrease in number of bacteria during holding, 66.7% of the foam samples gave a higher count after holding than before; the average increase in these samples was 176.3%. There was reduction in the bacterial content of the foam in vats when the covers were tightly closed, but in vats with the covers open or loose fitting there was an increase. At both beginning and end of the holding there were wide variations in bacterial contents of samples of foam from different parts of a vat. The butterfat content of foam varied from that of the corresponding milk and was higher in nearly all cases; at the end of the holding the percentage of fat in the foam usually was higher than at the beginning.

An investigation by Lomax and Clegg[61] at several pasteurizing plants where the milk had given unsatisfactory phosphatase tests showed that the trouble was due to excessive foam on the milk. It was 4 to 6 in. deep and 7 to 10 F lower in temperature than the milk.

Cooling After Pasteurization. The necessity of cooling market milk and cream to a low temperature immediately after pasteurization generally is appreciated and is demanded by many regulations. Pasteurization destroys a large percentage of the bacteria present but cannot prevent growth of those remaining or of those that gain entrance during the subsequent handling. Activity of such organisms must be prevented by low temperatures and, from the standpoint of control of bacterial growth, these should be reached quickly.

Ayers and Johnson [62] found that the sudden chilling of organisms surviving pasteurization has no effect on them. Milk was pasteurized in sterile flasks at either 145 or 160 F for 30 min and then cooled rapidly by running it through a sterile copper coil surrounded by brine; in each trial bacterial counts before and after cooling were essentially the same.

Home Pasteurization. It sometimes is desirable to pasteurize milk in the home and several types of home pasteurizers are available. Thomas [63] tested four commercial home pasteurizers for temperature by suitably placed thermocouples, and also for phosphatase destruction, and included tests on milk heated in an open and a covered sauce pan and in a covered double boiler. Results indicated that insufficient heating of the air and container above the milk level may result in a positive phosphatase test on the milk which comes in contact with the rim. Suggested solutions for the problem are to use higher temperatures or completely surround the milk container with hot water. Home electric pasteurizers have been found to give satisfactory results,[64] including the destruction of inoculated *Brucella* organisms.[65]

Bacteria Surviving Pasteurization

When pasteurization was being extended rapidly in the field of dairying, the prevailing idea was that bacteria which survived the process were largely spore-forming species. This view came, in part, from studies on the organisms present in milk heated to temperatures higher than those now employed in pasteurization. Investigations have shown that various nonspore-forming bacteria resist exposures accepted as satisfactory; they constitute a much larger percentage of the flora of pasteurized milk than do the spore formers and play an important role in the bacterial changes in pasteurized milk. Ayers and Johnson [62] found that of 225 unselected cultures which survived pasteurization at 145 F for 30 min, only 3 (1.35%) formed spores. Macy [66]

reported that, among 81 cultures isolated from laboratory pasteurized milk, there were 13 (16%) spore formers.

Factors Influencing Heat Resistance of Organisms. Resistance of a bacterial species to heat is not a sharply defined character that can be determined exactly. Variations in resistance occur with different cultures belonging to a species and also with the individual organisms in a culture. The relatively resistant cells in a culture may permit it to survive comparatively high exposures. In addition to the normal variation in heat resistance, certain factors have a definite influence on it.

Hydrogen-ion Concentration. Mattick and Nichols [67] studied the effect of the *p*H of milk on destruction of organisms by heat. Each sample was well mixed and divided into 5 portions. One portion was used as a control and small amounts of sodium hydroxide or hydrochloric acid were added to the others. After the samples had come to equilibrium, the *p*H values were determined and the samples pasteurized at 145 F for 30 min. Each sample was plated immediately before and after pasteurization. Variations in the *p*H values resulted in differences in destruction of organisms by the heat. As the *p*H values decreased, the numbers of bacteria surviving also decreased. The effects were much greater with some samples than with others.

Age of Bacterial Cells. Various investigations show that young bacterial cells are more easily killed by heat than older cells.[68-71] Sherman *et al.*[72] reported that milk held at temperatures permitting bacterial growth, such as 59.9 F, showed a greater percentage destruction of bacteria on pasteurization than similar milk when fresh or when held at temperatures, such as 40 F, that are too low for active multiplication of the bacteria involved. Hammer and Hussong [73] noted that young cells of certain organisms producing ropiness are more easily destroyed at pasteurization temperatures than old cells. With *E. coli,* Elliker and Frazier [74] found that during the period of most active reproduction at both 82.4 and 101.3 F the heat resistance declined to its lowest point. As the cultures entered the maximum stationary growth phase, their heat resistance increased.

In connection with the age of bacterial cells, the growth temperature is important. Commonly, organisms are more heat resistant when grown at their optimum than when grown at lower temperatures. With certain organisms, however, growth at temperatures below the optimum apparently results in increased resistance. A *Streptococcus* which showed increased resistance to heat as its temperature of growth was reduced below the optimum was described by Anderson and

Meanwell;[75] 2 cultures increased in heat resistance as a result of 24 hr storage at 37.4 to 39.2 F. Claydon[76] found that, at 50 F, *S. lactis* cultures grew more slowly than at higher temperatures but attained higher thermal resistance; the highest resistance noted with a culture grown at 50 F was 13 min at 143 F.

Data indicating that young bacterial cells are killed more easily than older cells have led to attempts to obtain lower bacterial counts on pasteurized milk by holding the raw milk for a short period at a temperature relatively favorable for bacterial growth before the heat treatment. In general, these have not been successful; presumably, various factors result in relationships other than those expected on the basis of tests with certain pure cultures.

Fat Content. In tests on certain nonspore-forming bacteria of relatively low heat resistance, Nichols[77] found that an increase in the butterfat content of the medium failed to influence the heat resistance.

Special Considerations. There is some evidence that an exposure to heat slightly below that required to kill an organism may so modify it that one or more characters, especially physiological ones, are different from what they were originally. When results suggesting such a change are obtained, they often are not accepted and are attributed to contamination. However, variations in organisms have been noted as a result of various treatments, so that character modification as a result of an almost fatal exposure to heat might be expected.

In the course of pasteurization experiments with pure strains of coliform organisms inoculated into sterile milk, Katzin *et al.*[78] made post-pasteurization counts by means of the most probable number technic, using tubes of lactose broth. Heavy growth sometimes was noted without gas formation. A number of such growths proved to be gram-negative rods. A few reverted to lactose fermentation on cultivation, confirming their identity as coliform organisms. Other strains retained various degrees of impairment of lactose and dextrose fermentation during the short period of observation but otherwise were typical.

Thermophilic Bacteria. The thermophiles in dairy products belong to various genera and are not limited to spore-forming species. The history of a sample of milk largely determines the types of importance in it. Some thermophiles are capable of growth at LTLT pasteurization temperatures, or above; usually they grow only slowly although certain species may develop rapidly. In general, thermophiles represent plant rather than farm contamination of milk. They may come from equipment in which milk stands hot for extended periods, espe-

cially when milk stone has been allowed to accumulate. Because of thermophiles, heaters, vats, hot pipe lines, etc., should not be used for excessive periods without cleaning; residual foam remaining in vats is of special importance in this connection.

Ayers and Johnson [79] isolated a thermophile from pasteurized milk which they named *Lactobacillus thermophilus.* It grew rapidly at 150 F but not so rapidly at 145 or 122 F; at 86 F growth was very slow. The organism was destroyed by heating to 160 F for 30 min or 180 F for 2.5 min. Hammer and Hussong [80] reported the isolation of an organism, which they designated *Bacillus calidolactis,* that coagulated skim milk at approximately 159.8 F under commercial conditions. The milk was intended for drying and was held hot after pasteurization. The organism did not grow below 113 F. Rudig [81] isolated a thermophile that withstood 170 F for 20 min but not for 30 min.

Hansen [82] fed 11 cultures (9 species) of thermophilic spore-forming bacteria, grown in sterile skim milk, to guinea pigs. The animals increased normally in weight and none of the organisms produced any detectable toxic substance. It was concluded that the presence of thermophiles in market milk appears unimportant from the public health viewpoint.

Thermoduric Organisms. Although other organisms also are involved, the thermodurics in dairy products belong primarily to the genera *Micrococcus, Streptococcus, Microbacterium,* and perhaps *Sarcina.* The order of their importance varies in different investigations and is influenced not only by the sources of the samples but by the medium and incubation temperature used in plating. For example, Thomas [83] reported that thermoduric counts on yeastrel milk agar averaged 12 times as high with incubation 4 days at 86 F (30 C) as with 2 days at 98.6 F (37 C). Thermodurics often are present in large numbers in milk as it comes from the farm, and they are essentially a farm problem but also may be added to milk in the plant.

Genus *Micrococcus.* Micrococci, especially those producing yellow colonies, are common on plates poured with pasteurized milk. In various media these resist the usual pasteurization exposures.[84] Several species of micrococci have been identified among the thermodurics isolated from milk, including *M. luteus, M. candidus, M. varians, M. epidermidis,* and *M. caseolyticus.*[85, 86] Although some of these organisms may come from the udders, the numbers normally are small and external sources of contamination often add many more.

Genus *Streptococcus.* Early investigations indicated that heat-resistant streptococci are common. Ayers and Johnson [87] studied 139

cultures of streptococci isolated from cow feces, the udder and mouth of the cow, and milk and cream, and found that, with a 30-min exposure in milk, 89 (64.03%) survived 140 F, 46 (33.07%) survived 145 F, and 3 (2.56%) survived 160 F; all were destroyed at 165 F. The 22 cultures forming long chains were much less resistant to heat than the others. Sherman and Stark [88] investigated 294 cultures of streptococci which grew actively at 113 F and noted that each of the species included survived 30 min at 145 F in milk. More recent studies confirm the heat resistance of certain species of streptococci; these include *S. thermophilus, S. durans, S. bovis, S. faecalis,* and *S. paracitrovorus.*

Genus *Microbacterium.* Microbacteria are among the most heat resistant of the nonspore-forming bacteria and have been reported to survive 185 F, and in a few instances 194 F, for 10 min in milk. These organisms probably have been missed in certain studies because they may fail to grow at 98.6 F (37 C). *M. lacticum, M. liquefaciens,* and *M. flavum* have been encountered among the thermodurics isolated from milk.

Nashif and Nelson [89] invariably isolated microbacteria from 0.01-ml portions of pasteurized market milk and frequently from similar portions of laboratory pasteurized milk from individual producers. The few farm utensils examined did not contain them in detectable numbers. In no case did the organisms constitute an important percentage of the count on an unpasteurized product, but they sometimes made up more than half the count on a pasteurized product.

Other Groups. Mesophilic spore-forming bacteria may be encountered in pasteurized milk, the numbers usually being small because of the relatively few spores in raw milk; aerobes are more common than anaerobes. Some lactobacilli survive pasteurization [90] and may play a part in the ripening of certain cheeses. Organisms belonging to the genera that have little resistance to heat also appear to survive pasteurization occasionally although recontamination is another possibility.

Sources of Thermodurics. Numerous studies emphasize that poorly cleaned farm equipment is the common source of the thermodurics in milk. In such equipment many species of bacteria, including thermodurics, can grow and then contaminate the milk for which it is used. Soil, manure, bedding, plants, water, etc., are common habitats of thermodurics from which equipment can be contaminated. With adequate care of equipment, the thermoduric count of milk coming from a farm can be kept relatively low.

Milking machines that are not properly cleaned and sanitized fre-

quently are responsible for high thermoduric counts. Dotterrer [91] noted that milking machines caused high thermoduric counts more often than other equipment. Parfitt[92] found that, with 104 milk shippers involved, machine-drawn milk had much higher thermoduric counts than hand-drawn milk; also, there could be a striking build-up of thermodurics in a machine.

Bryan *et al.*[93] reported that properly pasteurized milk (phosphatase-negative) from a small dairy increased in count during a 4-month period from its normal level of 15,000 per ml to as high as 2,500,000. Despite proper plant sanitation, the high counts persisted. The immediate source of the high counts of heat-resistant bacteria was the milk from 1 of the 7 producers. An unclean milking machine contributed heat-resistant organisms directly to the milk and also served to inoculate the cows' udders with the organisms. After the equipment was properly cleaned and sanitized, the cows rid themselves of the heat-resistant bacteria in from 1 to 4 months. During this same period the plate count on the pasteurized milk decreased to its previous normal level.

Cordes [94] noted that over a 5-month period the laboratory pasteurized count of a plant receiving milk from 66 dairies was 28,360 per ml; with 50 dairies using milking machines the average count was 31,000 and with the 16 dairies using hand milking it was 1,550. Inflations and milk tubes gave pasteurized counts as high as 1,500,000 per ml when rinsed with 50 ml sterile water.

Addition of thermodurics to milk by a milking machine is controlled readily by proper care of the machine. Thomas *et al.*[95] found that unsterile machines often contributed over 100,000 thermodurics per ml of milk but that thorough cleaning immediately after milking followed by steam sterilization resulted in low counts.

Although pails, strainers, coolers, and other equipment on farms are of less importance than milking machines as a source of thermodurics, they sometimes are responsible for excessive numbers and should be considered whenever high thermoduric counts are obtained. Their significance is indicated by the fact that milk high in thermodurics may come from farms on which milking is done by hand.

Thermodurics may be added to milk in the processing plant through their accumulation in equipment because of faulty cleaning and sterilization, but adequate care controls them rather completely. Corash [96] noted that city handling of raw milk up to the storage tank is not a major factor in increasing the thermoduric level and that raw milk stored under proper refrigeration showed only a very slight increase

in 24 hr. Repasteurization may be responsible for a build-up of thermodurics in milk.

Milk stone may contain large numbers of thermodurics. Cordes [91] reported that 0.1 g milk stone from a can in 5 ml sterile water gave a pasteurized count of 1,080,000 per ml. Thomas *et al.*[97] found micrococci and sarcinae and also microbacteria especially numerous in milk stone. With sufficient heating, the numbers of organisms in milk stone are small.

Low Bacterial Efficiencies. With some samples of milk, pasteurization gives low bacterial efficiencies due to the presence of considerable numbers of thermodurics. This situation was noted early by Dotterrer [98] who occasionally found high bacterial counts on milk from certain pasteurizing plants. With milk from individual farms he noted great variations in the percentages of organisms destroyed by pasteurization and in the numbers per milliliter of the pasteurized milk.

Hussong and Hammer [99] studied the heat resistance of the organisms in milk from various farms by pasteurizing small amounts in sealed tubes at 142 F for 30 min. Wide variations were noted in the efficiencies obtained, and there also were variations with the samples from a given farm. Milk from one farm showed low efficiencies and high counts on the pasteurized milk over an extended period. Better methods of producing and handling the milk resulted in lower initial counts and higher efficiencies, so that counts on the pasteurized milk were markedly decreased; when the initial counts then increased, owing to hot weather, the higher efficiencies and lower counts on the pasteurized milk persisted. Considering the samples from the various farms, there was no close relationship between efficiencies and initial counts.

Many plants have experienced high bacterial counts on pasteurized milk. With bacterial standards for pasteurized milk, this has stimulated improvements in the methods of milk production, especially in the care of milking machines and general equipment, and has been an important factor in the establishment of excellent milk supplies in many areas.

Effect of Cooling on Thermodurics. Thomas *et al.*[100] determined the thermoduric counts on monthly or fortnightly milk samples from 171 farms. The farms were classified into 4 groups according to efficiency of milk cooling and sterilization of utensils during the summer months. Inadequately cooled milk did not have higher thermoduric counts when sterilization of the utensils was good, whereas

efficiently cooled milk did not have lower thermoduric counts when sterilization was poor.

Effect of Homogenization on Thermodurics. Homogenization would be expected to increase the thermoduric count through the breaking up of bacterial clumps. Corash [96] found with 48 samples that homogenization increased the thermoduric count from 3.5 to 34%; in another series of 55 samples the average increase was 64%.

Detection of Thermodurics. Since raw milk sometimes contains excessive numbers of thermodurics, milk from individual herds often is tested for heat resistance of the flora. Samples can be laboratory pasteurized in tubes with the LTLT method and plate counts made before and after, or only after, heat treatment. Counts also can be made with the Burri technic or the oval tube technic. Pasteurization of many small portions of milk with the HTST method presents difficulties, unless relatively large errors in the exposure are accepted.

A microscopic procedure for detecting thermodurics in milk was suggested by Mallman *et al.*[101] With it, 5 to 10 ml of milk is held at 136.4 to 140 F for 2 hr and a microscopic count made. Samples showing 40,000 or more bacteria per ml are considered to contain excessive numbers of thermodurics. The authors noted that microscopic counts on pasteurized milk directly after pasteurization represent living and dead bacteria; the incubation destroys nonthermoduric organisms and causes their dissolution so that a microscopic count after the incubation represents viable thermodurics.

Bryan and Bortree [102] determined the presence of heat-resistant bacteria in milk by pasteurizing the milk-agar mixture after making a Frost little plate and then making a second little plate for the pasteurized milk count. Results compared favorably with those of the pasteurization test in a series of 1,375 samples; they were ready within 5 hr.

In a comparison of various methods of detecting thermoduric organisms in milk, Fischer and Johns [103] found that the oval tube technic gave results in much closer agreement with the plate count than did the microscopic procedures tested. Macy and Erekson [104] reported a close correlation between results of the Burri technic and the plate count. Parfitt [92] noted that, in general, there is no relation between methylene blue reduction and the thermoduric population of milk. He considered that one of the reasons for high thermoduric counts is that milk producers have found short cuts in methods which are not detected by the methylene blue test.

Resistance of Thermodurics to Chemicals. In the treatment of utensils responsible for high thermoduric counts, the results of Maack and Prucha [105] are of significance. They noted that heat-resistant organisms also were resistant to chlorine. One nonspore-forming species that required 143 F for 3.5 hr to kill all the bacterial cells required 100 ppm chlorine for 2 min or 20 ppm for 5 min for its destruction. Using hypochlorites for sterilization of equipment, Thomas *et al.*[106] found it was necessary to have clean smooth surfaces and in practice this method could not be relied on to give the same degree of sterility as steam. When hypochlorite sterilization was used only 1 or 2 days a week for short, irregular periods, there was a marked build-up of thermodurics in utensils. The predominant types of bacteria surviving steam and hypochlorite sterilization have been reported to be microbacteria, spore formers, and micrococci.[107]

Significance of Thermodurics. Corash [96] noted that thermodurics have no significance from the viewpoint of pathogenicity but their presence in milk in large numbers indicates an unsatisfactory production environment. The heat resistance of thermodurics definitely differentiates them from the pathogens that occasionally are spread through milk.

Survival of Coliforms. Although coliform organisms commonly are destroyed by pasteurization, some cultures survive under certain conditions, especially when the initial numbers are large. Heat resistant coliforms may be important in connection with coliform standards for pasteurized products; they also appear to have caused gas in cheese made from pasteurized milk.

Ayers and Johnson [108] studied the heat resistance of 174 coliform cultures isolated from cow and human feces, flies, milk, cream, and cheese and noted that 12 (6.89%) survived 145 F for 30 min. Tanner and Windsor [109] found 1 of 23 cultures of *E. coli* repeatedly survived heating in sealed tubes at 145 F for 30 min. In the examination of 100 samples of milk, Beavens [110] reported that with 32 of them *Escherichia-Aerobacter* organisms survived the temperatures used in commercial pasteurization. Stark and Patterson [111] studied 505 cultures of *Escherichia-Aerobacter* organisms recently isolated from water, raw and pasteurized milk, and human and bovine feces. Of these, 487 (96%) failed to survive 140 F for 30 min; 14 of the remaining 18 were destroyed at 143 F for 30 min; and the other 4 were destroyed at 145 F for 30 min.

Long *et al.*[112] found that *Escherichia* cultures of comparatively high heat resistance were rather frequently encountered in dairy products,

especially butter. Of 116 cultures selected from isolations from butter, 74 (63.8%) survived 20 min at 143 F, 48 (41.4%) survived 30 min, and 28 (24.1%) survived 40 min. Counts on certain of the resistant cultures indicated that in some of them appreciable numbers of cells still were present after 50 min at 143 F. Heat-resistant *Aerobacter* cultures were not encountered. The authors suggested that the heat treatment given churns in cleaning and sterilizing may tend to select the more resistant types of coliform organisms which then multiply and are added to butter during the churning process. Craige [113] reported that 8 strains each of *Escherichia*, intermediate, and *Aerobacter* coliforms isolated from raw milk, pasteurized milk, and ice cream all survived 143.6 F for 30 min in milk when present in sufficient number; no strain tested survived when the concentration before pasteurization was <700 per ml. He considered that one of the causes of coliforms in pasteurized milk is excessive numbers in the raw milk. Swartling and Lindgren [114] noted that 6 of 10 coliforms tested gave indications of survival in milk heated at 157.1 F for 16 sec. Three of the cultures were isolated from HTST pasteurized milk, and the other 3 were obtained from a type culture collection.

Coliform tests on pasteurized milk are useful in detecting recontamination. Apparently, heat-resistant coliforms are unusual in raw milk produced under good conditions and, with proper processing, coliforms are rarely found in the pasteurized product. Buchbinder and Alff [115] concluded that so-called heat resistant coliforms are of no practical significance in the coliform test on pasteurized milk. With large numbers of coliforms in the raw milk, there definitely is a greater chance of some surviving pasteurization than when the numbers are small. Coliforms obtained from pasteurized milk can be tested for heat resistance if their origin becomes important.

In connection with coliforms in pasteurized milk, the results of Dahlberg [116] are significant. He found that, in pasteurized milk held at refrigeration temperatures, the coliforms increased more rapidly than the total bacterial count. In freshly pasteurized milk <0.02% of the total bacteria were coliform types. After 4 days at 35 to 40 F in October, the percentage of the total bacteria that were coliforms did not increase, but in July and August it increased to 1.12%. After 4 days at 45 to 50 F or 55 to 60 F in October, the coliforms constituted about 5% of the total count; in July and August at 45 to 50 F they constituted 88%, and at 55 to 60 F they constituted 50%. Dahlberg suggested that, when they are contaminants, the coliforms may grow

relatively rapidly because they have not been subjected to the heat treatment and are actively growing organisms.

Comparative Bacterial Counts After HTST and LTLT Pasteurization. Most investigations indicate that, on the average, destruction of organisms in milk is somewhat less with HTST than with LTLT pasteurization. Differences in destruction vary widely from sample to sample and sometimes bacterial counts actually are lower with the HTST method.

In a comparison under commercial conditions of 160 F for 15 to 20 sec and 143 F for 30 min on 6 lots of milk, Quinn and Burgwald [117] obtained the following average values: Raw milk 1,066,667 per ml; pasteurized, HTST 50,271 (95.28% reduction), and LTLT 35,087 (96.71% reduction). In laboratory tests, holding milk at 160 or 162 F for 25 to 60 sec did not increase the bacterial efficiency of the process; the creaming ability was decreased.

With 15 samples of milk, Hileman *et al.*[118] obtained average counts after pasteurization as follows: 143 F for 35 min in the laboratory, 13,000 per ml; 161 F for 16 sec in the laboratory, 17,533; 161 F for 16 sec in the plant, 30,200. The higher counts obtained with HTST pasteurization were considered to be due largely to certain *Micrococcus* species surviving the process. In the trials of Hileman and Leber [119] on 125 samples of milk, average counts on the pasteurized milk were as follows: 143 F for 35 min in the laboratory, 11,168 per ml; 161 F for 16 sec in the laboratory, 13,761; 161 F for 16 sec in the plant, 25,932.

Millenky and Brueckner [120] found that milk had to be pasteurized at approximately 165 F in an electric HTST pasteurizer to equal the bacterial destruction obtained with the LTLT method. When the flora was not particularly resistant, temperatures below 165 F resulted in a satisfactory bacterial destruction.

The following results were reported by Fay: [121] Of 262 lots of milk from 4,000-gal storage vats pasteurized simultaneously by the HTST method at 162 F for 16 sec and by the LTLT method at 143 F for 33 min, 55% gave higher plate counts after the HTST process. Of 40 samples giving essentially the same counts after the two methods, the tendency was toward a higher count after the HTST process. Results were similar for (1) milk having low initial counts of heat-resistant organisms, (2) milk having high initial counts of heat-resistant organisms, and (3) milk having high total counts (over 1 million per ml).

Ashton [122] reported higher thermoduric counts with HTST than with LTLT pasteurization, but the keeping quality of the latter milk

was inferior. The difference in keeping quality suggested fundamental differences in the effects of the two time-temperature combinations.

General Considerations

Comparative Growth of Bacteria in Raw and Pasteurized Milk. Pasteurization of milk kills the types of bacteria which grow most readily at relatively low temperatures. Accordingly, at such temperatures multiplication of bacteria is controlled more easily in pasteurized than in raw products, particularly if the latter have been produced carelessly. The increased period that pasteurized milk will remain satisfactory cannot be predicted because of various influencing factors, but at the temperatures used for holding milk it is substantial.

Powell [123] collected raw cream and flash pasteurized small portions in a laboratory apparatus at 155, 165, 175, and 185 F, after which they were held at 35 F for 10 days. In the 12 trials, reduction in bacteria during pasteurization increased with an increase in temperature. The raw cream showed an increase in count during holding at 35 F for 10 days, whereas counts on the pasteurized portions remained essentially as they were originally. In the fresh raw cream, acid-coagulating bacteria predominated but as the cream aged they were gradually replaced by the slower-growing peptonizers and acid formers. In the pasteurized cream the percentage of bacteria that were peptonizers increased as the heating temperature was raised and these organisms predominated at 165 F, or above.

Sherman *et al.*[124] noted that at 32 F bacterial growth in pasteurized milk was much slower than in raw milk. Uncontaminated pasteurized milk kept 2 to 3 times as long as raw milk of substantially the same quality or bacterial count. Good-quality raw milk usually kept about 4 weeks before spoilage, whereas a comparable quality of pasteurized milk usually kept in excess of 8 weeks. Inoculation of pasteurized milk with a minute amount of raw milk decreased its keeping qualities to a point substantially the same as that of raw milk. In the trials of Nicholas and Anderson,[125] pasteurized or homogenized-pasteurized milk with an initial bacterial count from 1,000 to 94,000 per ml kept in an electric refrigerator at an average temperature of 40 F for an average period of 17.2 days before detectable spoilage occurred. Two lots of raw milk stored under the same conditions remained unspoiled 4 and 7 days, respectively.

Fermentation in Pasteurized Milk. At one time it often was assumed that fermentations in pasteurized and raw milk are essentially

the same. This probably was the result of using inadequate pasteurization exposures and/or permitting contamination of the heated milk. The relatively rapid production of acid that is so common when raw milk undergoes bacterial changes greatly limits the growth of acid-sensitive species. It does not occur with normally pasteurized milk, and various organisms have a greater opportunity for growth. Many

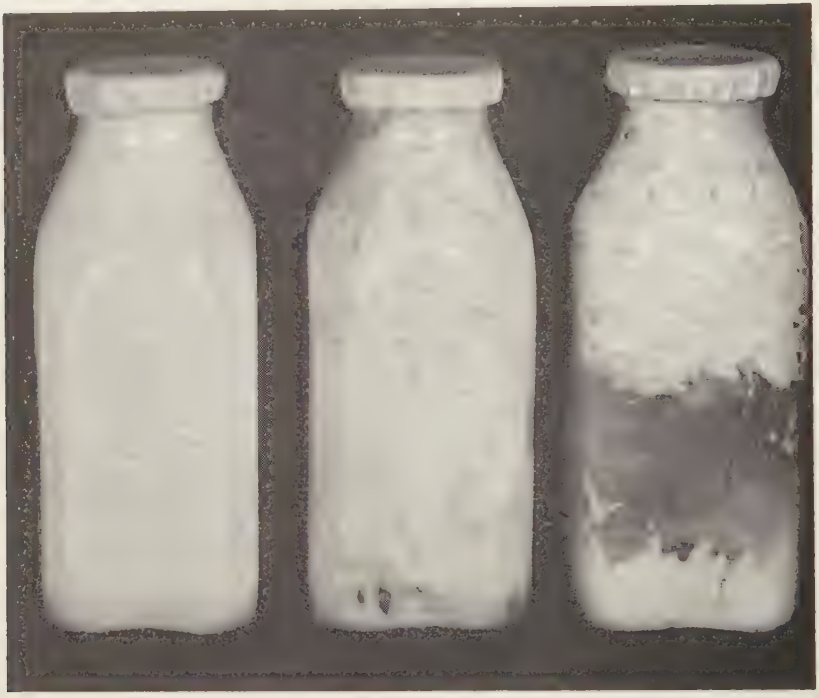


Figure 25. Fermentations in different samples of pasteurized milk after 2 days at 75 F.

consumers have complained that pasteurized milk does not sour but shows more objectionable changes.

The fermentation that takes place in pasteurized milk shows variations, just as does the fermentation in raw milk. It is influenced by such factors as contamination subsequent to the heating, holding temperature, original flora, etc. In general, coagulation occurs, due to either sweet curdling or acid formation. Coagulation due to acid production is not noted as regularly as with raw milk and, when it takes place, microscopic examinations or plate cultures often show a variety of organisms, instead of the predominance of streptococci that

is so common when raw milk develops acid. Although acid-forming organisms may be present in pasteurized milk, at relatively low temperatures their action is slower than the action of the acid formers in raw milk. The acid-forming streptococci that survive pasteurization commonly are types requiring relatively high temperatures for active development. Thurston and Olson [126] reported that the pasteurization of low-count milk at 145 F for 30 min changed the flora so that, on storage, acid developed slowly and coagulation occurred at low acidities, apparently because of the production of a rennin-like enzyme by the bacteria.

Following coagulation of pasteurized milk, due to production of an enzyme or acid, other changes occur. There may be active digestion of the milk with formation of objectionable odors through continued activity of the organisms involved in the sweet curdling. Production of gas is common; spore-forming anaerobes may be involved although in some cases coliform types that resisted the pasteurization, or gained entrance to the milk following the heat treatment, are responsible. An off odor that often is noted in stored pasteurized milk in the United States is that caused by *Ps. fragi;* presence of the organism presumably is due to recontamination.

Advantages of Pasteurization. The advantages of proper pasteurization are (1) reduction in spread of pathogens through milk and (2) improvement in its keeping quality.

1. Reduction in Spread of Pathogens. Pasteurization destroys the pathogens likely to be spread through milk. It is no guarantee against future infection, however, and epidemics have been due to contamination of milk after heating. In 1923, Godfrey [127] concluded from a study of a large number of milk-borne epidemics that 85 to 90% were due to farm infection. At present, with the extensive use of pasteurization, farm infection of milk with pathogens is largely concealed.

The actual decrease in milk-borne diseases as a result of pasteurization cannot be determined because other factors also have been operative. These include improvements in water supplies, suitable sewage disposal, immunization, etc., and it is impossible to ascertain the influence of these separately. The value of pasteurization is shown directly by instances in which raw milk from a certain lot has caused an epidemic, while pasteurized milk from the same lot has not. Evidence of the value of pasteurization also is provided by the definite decrease in milk-borne diseases that has occurred in certain cities as a result of compulsory pasteurization; often such pasteurization has followed a severe milk-borne outbreak.

Extensive use of pasteurization permits the consumption of milk almost anywhere in the United States with full confidence in the safety of the product, a situation that did not obtain when raw milk commonly was consumed. Pasteurization is used widely with certified milk which is produced under the most rigid conditions practical. Failures sometimes occur in milk production methods, even when all normal precautions are taken; proper pasteurization corrects for these and in this way has revolutionized the dairy industry.

2. Improvement in Keeping Quality. The necessity of delivering milk with reasonably good keeping quality is appreciated by all milk dealers. A product that readily sours or undergoes other changes is certain to bring complaints and probably loss of patronage. Milk should have a keeping quality that enables it to remain in a satisfactory condition under normal refrigeration for any reasonable period, and this is attained most readily by pasteurization. Although the primary object of pasteurization is to provide safe milk, the other advantages need not be ignored.

Objections That Have Been Raised to Pasteurization. Various objections have been raised to the pasteurization of market milk. At one time there may have been some foundation for certain of these, but improvements in pasteurization methods and in their control, and also in the quality of the raw milk, have removed any basis for them, and objections now come largely from persons who do not appreciate what the process accomplishes.

Objections to pasteurization which have been most frequent are:

1. Organisms Developing in Pasteurized Milk Form Harmful Products. This idea appears to have been based on the work of Flügge,[128] who produced illness in puppies by feeding them milk cultures of organisms obtained from boiled milk. The results are not applicable to pasteurized milk, since the organisms were isolated from milk heated to much higher temperatures than those used in pasteurization, but the species which survive boiling also would survive pasteurization. With long holding at temperatures favorable for bacterial growth, either raw or pasteurized milk may contain products that are toxic for experimental animals, but ordinarily the milk has undergone conspicuous changes and would not be used.

2. Infants Do Not Develop Well on Pasteurized Milk. Occasionally, the claim is made that pasteurized milk is not satisfactory for infants. The best argument against this is the large number of well-developed children who have been fed the product. Observations in

various cities show that infants make entirely normal gains on pasteurized milk.

Convincing evidence of the value of pasteurized milk for children was obtained by Frank *et al.*[129] in a field study. Because practically all the children had received some heated milk, the raw-milk group included those children who had received raw milk for more than half their lives, including the latter half, whereas the heated-milk group included those children who had received heated milk for more than half their lives, including the latter half. Results on more than 3,700 children from 10 months to 6 yr of age showed that there was no significant difference between the average weights or heights of the two groups. However, parents of children receiving predominantly raw milk reported a higher incidence of diphtheria, scarlet fever, intestinal disturbance, and rickets than did parents of children receiving predominantly heated milk.

3. Pasteurization May Be Used to Mask Low-quality Milk. When pasteurization was less common than at present, it undoubtedly sometimes was employed to give milk which has undergone extensive bacterial growth a keeping quality that made it marketable. However, milk that is to be pasteurized requires the same supervision as milk that is to be sold raw and bacterial standards for it regularly are used.

4. Milk Enzymes Are Destroyed. The normal milk enzymes are partially or completely destroyed by pasteurization. However, there is no evidence that these enzymes play a part in the digestion process, and any influence that pasteurization has on them probably is unimportant from the standpoint of the person consuming the milk. Destruction of lipase by pasteurization is an advantage with those lots of milk that would become rancid if left in a raw state.

5. Influences the Composition of Milk. Investigation indicates that pasteurization at the usual exposures has little effect on the composition of milk. Rupp [130] analyzed milk samples pasteurized at various temperatures for 30 min. Milk heated to 145 F did not undergo an appreciable change; the soluble phosphates of lime and magnesia did not become insoluble. At 155 F the quantities of phosphoric acid, lime, and magnesia in the serums of raw and pasteurized milk were practically the same. The albumin did not coagulate at 145 F, but, at 150 F, 5.75% was rendered insoluble, and as the temperature was increased the amount of coagulated albumin increased. Elvehjem [131] reported that from work with dogs there was no indication of a reduced availability of calcium and phosphorus in pasteurized milk.

6. Influences Vitamins. With an appreciation of the value of

the vitamins in milk, the question of the influence of pasteurization on them became of importance. It is now recognized that the type of pasteurization, condition of equipment, etc., influence the results. There are differences in the vitamin contents of raw milk as a result of season, feed, condition of the animal, and method of handling, and these may be of more significance than the effect of pasteurization.

Elvehjem [131] noted that pasteurization does not lower the vitamin A or carotene content of milk, and there is no indication of loss of vitamin D but there may be some destruction of the other fat soluble vitamins, E and K.

Woessner *et al.*[132] found that pasteurization by the LTLT method caused a 20% loss of ascorbic acid. They noted that the advantage of HTST pasteurization in preserving ascorbic acid seems dependent upon the fact that the destruction of ascorbic acid appears to be related more to the period it is held at the elevated temperature than to the temperature itself. The most serious losses in antiscorbutic activity during processing and delivery of milk were caused by copper contamination and exposure to light. At irregular intervals during 18 months, Holmes *et al.*[133] pasteurized 20 lots of milk with the HTST process. The average values obtained just before and immediately after pasteurization were: Ascorbic acid, 16.4 and 16.6 mg per liter, respectively; riboflavin, 1.50 and 1.50 mg; and thiamin, 0.36 and 0.35 mg. Elvehjem [131] noted that pyridoxine and nicotinic acid are very stable and no loss of these factors on pasteurization would be expected; also, preliminary results indicate pasteurization does not inactivate pantothenic acid.

With LTLT pasteurization in a stainless steel vat, Holmes *et al.*[134] obtained the following effects on vitamins: Ascorbic acid, raw milk (32 samples) 19.7 mg per liter, pasteurized milk (32 samples) 15.9 mg; riboflavin, raw milk (31 samples) 1.51 mg, pasteurized milk (31 samples) 1.48 mg; thiamin, raw milk (29 samples) 0.33 mg, pasteurized milk (31 samples) 0.30 mg. Holmes [135] reported that pasteurization by the LTLT and the HTST processes showed the following effects on riboflavin: 24 lots LTLT pasteurization (stainless steel, spray vat), 1.46 mg per liter before and 1.43 after; 12 lots LTLT pasteurization (stainless steel, coil vat), 1.52 mg before and 1.49 after; 10 lots HTST pasteurization, 162 to 181 F for 15 sec (depending on directions of manufacturer), 1.41 mg before and 1.42 after.

Theophilus and Stamberg [136] reported that neither pasteurization, homogenization, nor storage for 24 hr at 40 F in a dark refrigerator decreased the riboflavin content of milk. As much as 40% of the ribo-

flavin in milk was destroyed by 2 hr exposure to direct sunlight in clear glass bottles even though the milk was quite cool; good shade, brown glass bottles, or paper containers gave good protection.[137]

Special Temperature and Time Relationship. The shift to HTST pasteurization, with the general advantages and no serious disadvantages, suggests the possibility of an additional increase in temperature and corresponding decrease in time. Holland and Dahlberg[138] reported that a point was found at 170 F which appeared especially promising as a new standard for pasteurization. Later, Dahlberg *et al.*[139] conducted experiments under commercial conditions in which the heat treatment given the milk consisted of a controlled period above 140 F during which the milk was heated to the desired temperature and again cooled below 140 F; there was no definite holding at the highest temperature attained. The method was designated quick-time pasteurization. Milk was successfully quick-time pasteurized at 177.5 to 169 F, with the period above 140 F varying from 5 to 24 sec. Milk was pasteurized at 170 F with 12 sec as the total period to heat and cool.

Using a small-tube heat exchanger, Tobias *et al.*[140] found that 168.34 F for 2.36 sec gave a destruction of the test organism equivalent to laboratory pasteurization at 143 F for 30 min. A temperature of 169.7 F was necessary to give a satisfactory reduction in phosphatase activity.

Davies[141] studied a machine consisting of a stainless steel cylinder into which is inserted an electric heating element capable of radiating heat as high as 1350 F. Heat radiates from the element through the air to the thin film of milk flowing on the inside surface of the cylinder; it takes about 1 sec to travel from the top of the cylinder to the bottom. Since the cylinder wall always remains cooler than the milk that is traveling down it, there is no adhering of milk solids. The length and diameter of the cylinder and distance of the heating element from the film of milk can vary with the capacity of the machine, type of product, etc. The process involves high heat transfer and requires instantaneous flash cooling. Actual operation of the machine showed the following: (1) Milk entering at 55 F and subjected to 1350 F will emerge from the machine at about 185 F; (2) milk entering at 100 F and subjected to 1000 F will emerge at 200 F; and (3) milk entering at 75 F and subjected to 1300 F will emerge at 205 F. At 1000 F, only 0.1 sec is required to kill organisms. Bacterial counts on the raw milk varied from 2,400 to 640,000 per ml. The lowest

count obtained was 14 per ml using 1300 F. The average lethal efficiency was consistently 99.95% and higher.

Using equipment consisting essentially of a stainless steel pressure tank and small bore stainless steel tubing which is heated by high-amperage, low-voltage alternating current connected to the tubing at three or more points, Hankinson *et al.*[142] studied the possibility of increasing the pasteurization temperature to a point where the "come-up" time (time to heat milk to a given temperature) is sufficient to ensure adequate pasteurization, the holding time thus being eliminated. Preliminary studies indicated that there was destruction of phosphatase and 100% kill of a 24-hr culture of *E. coli* within the range of 176 to 185 F at heating rates varying from 170 to 1700 F per sec. The authors suggest that if further studies show that the method is effective at temperatures below 200 F, it could be applied to existing HTST equipment by eliminating the holding tube and raising the cut-out temperature for the flow-diversion valve.

Electrical Treatment of Milk

Studies concerning the effect of an electric current on bacteria have included attempts to destroy most of the organisms in milk by passing a current through it. In the investigations an important point has been whether the effect on the organisms is due to the current or the heat generated by it.

Beattie and Lewis[143] found that milk could be rendered free of *E. coli* and *M. tuberculosis* by an electrical method without raising the temperature higher than 145.4 to 147.2 F. The temperature effect was very short and was not the principal factor in the destruction of bacteria. The keeping property of the milk was increased and the taste was not altered. Later, the investigators[144] again concluded that heat is not the main factor in the destruction, but that the electric current is an important agent; bacteria were destroyed at temperatures much below their thermal death points. Anderson and Finkelstein[145] studied the electrical treatment of milk with special equipment; it gave satisfactory destruction of bacteria and this appeared to be due to the heat produced.

Prescott[146] investigated an installation for treating milk in which the milk was heated to about 118 F in a regenerative cooler and then subjected to an alternating current of 220 volts, 60 cycles. In the treatment chamber the milk was raised to 158 to 160 F and then cooled. The milk did not have a cooked taste, the cream line was well marked, and the keeping quality was excellent. During the

winter, when the raw milk had a count of <200,000 bacteria per ml, the percentage reduction in the organisms approximated 90%; in the warmer months, with the raw milk running from 500,000 to 600,000 per ml, the reduction varied from 97 to 98.5%. *M. tuberculosis* was destroyed.

In laboratory trials Gelpi and Devereux [147] found a much lower destruction of bacterial spores added to milk when pasteurizing at 145 F for 30 min than when subjecting the milk to an electrical treatment in which 35 to 40 sec were required to pass through the machine, 10 to 14 sec being the period the temperature was at 159.8 F. With the various species the percentage reductions with pasteurization were 0 to 13.0%, whereas with the electrical treatment they were 71 to 99.9%. In further studies the investigators [148] obtained results which indicated that destruction of endospores by an electrical process is not due entirely to the heat created in the medium surrounding the spores but also to another heat factor, namely, the heat generated within the spores.

Effect of Ultraviolet Rays on Bacteria in Milk

Various trials indicate that some of the organisms in milk can be destroyed by ultraviolet rays.

Houghton and Davis [149] investigated the action of ultraviolet rays on bacteria in various materials when flowing through a trough exposed to a lamp. With milk, a comparatively low reduction in the organisms was obtained. The ultraviolet rays had a tendency to kill the undesirable organisms present, leaving *S. lactis* in the majority. There was no perceptible change in the flavor of the milk.

Ayers and Johnson [150] made tests in which milk was exposed in various ways to ultraviolet rays generated by a quartz mercury-vapor lamp. The most satisfactory method of exposure was over two revolving drums, the tops of which were 4 in. below the light tube. The milk picked up by one drum was exposed, then collected by a scraper, after which it was picked up by the second drum and again exposed; finally, it was collected by a second scraper. When the milk was exposed in thin layers there was a marked reduction in bacteria and this was independent of the action of the heat, since the temperature of the exposed milk never was above 86 F. The two most important factors in the success of the method were thickness of the layer of milk and length of exposure. Results on 2 samples indicated that the rays did not have a selective action on organisms. When milk was exposed under conditions suitable for satisfactory

reduction of bacteria by the rays, a disagreeable flavor was produced.

Weckel [151] reported that radiation of whole milk of high quality under commercial conditions appears to have little influence on the bacterial counts, but that with milk of poor quality the counts are reduced. Radiation did not have a selective action on organisms occurring naturally in milk or on such species as *S. lactis, E. coli,* and *B. coagulans* when introduced into the milk. In tests with an ultraviolet vitaminization apparatus, Sauter [152] found that, with a flow of 2,000 liters per hr, about 70% of the pre-vitamin was transformed to vitamin D and the decrease in bacterial count amounted to 60 to 80%.

Supplee *et al.*[153] noted that the lethal effectiveness of ultraviolet energy applied to smooth-flowing milk films is dependent upon intensity and special characteristics of the radiation employed. Percentage reduction in bacterial counts on raw mixed milk in excess of 90% and, frequently, up to 95 to 98% may be obtained with a high degree of regularity by preferred methods of applying ultraviolet radiation of appropriate intensity and spectral characteristics.

Nicholson [154] found that ultraviolet rays do not penetrate milk and described an apparatus in which milk in a $\frac{1}{64}$ in. film flows over cylinders, arranged in pairs, which rotate away from each other. A baffle removes milk at the contact point of the cylinders. Film thickness is controlled by the rate of delivery of milk to the top pair of cylinders. A fan removes ozone. Germicidal lamps can be swung aside during cleaning. Milk with a bacterial count of $<2{,}500$ per ml was obtained with the apparatus.

Effect of Ultrasonic Waves on Bacteria in Milk

Ultrasonic waves have been suggested as a means of destroying bacteria in various materials, including dairy products. Jacobs and Thornley [155] found that organisms varied in rate of destruction by ultrasonic waves; death rates for cocci were lower than for rod-shaped organisms. Nature of the suspending liquid affected survival considerably. Under conditions which caused *E. coli* in broth to die rapidly, the organism in whole milk was unaffected. Dried skim milk and casein solutions also afforded protection, the extent depending on the concentration. Bacterial reductions of 50% were obtained in milk with waves of certain intensities by Tobler.[156] Acid formation was slower, perhaps due to the reduction in organisms. There was no effect on creaming, curdling, or phosphatase activity.

REFERENCES

1. *Conn. (Storrs) Agr. Expt. Sta. 14th Ann. Rept.*, 105 (1901).
2. *Conn. (Storrs) Agr. Expt. Sta. 15th Ann. Rept.*, 33 (1902–1903).
3. *J. Infectious Diseases*, **7**, 47 (1910).
4. *Mich. Agr. Expt. Sta. Tech. Bull.* 84 (1927).
5. *S. Dak. Agr. Expt. Sta. Bull.* 257 (1931).
6. *Food*, **19**, 25 (1950).
7. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 104 (1924).
8. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 389 (1917).
9. *Ia. Agr. Expt. Sta. Research Bull.* 28 (1916).
10. *Ia. Agr. Expt. Sta. Research Bull.* 47 (1918).
11. *Mass. Agr. Expt. Sta. Bull.* 187 (1918).
12. *Ia. State Coll. J. Sci.*, **4**, 181 (1930).
13. *Mich. Agr. Expt. Sta. Spec. Bull.* 335 (1946).
14. *J. Dairy Sci.*, **23**, 503 (1940).
15. *Milk Dealer*, **41** (3), 48 (1951).
16. *J. Dairy Sci.*, **1**, 272 (1917).
17. *J. Am. Vet. Med. Assoc.*, **49**, 678 (1916).
18. *Ia. Agr. Expt. Sta. Bull.* 59 (1901).
19. *U. S. Public Health Marine Hospital Service Hyg. Lab. Bull.* 56, 739 (1909).
20. *J. Dairy Sci.*, **1**, 498 (1918).
21. *J. Agr. Research*, **31**, 695 (1925).
22. *J. Dairy Sci.*, **26**, 295 (1943).
23. *U. S. Dept. Agr. Yearbook*, 431, 1895. U. S. Government Printing Office, Washington, D. C.
24. *J. Milk and Food Technol.*, **12**, 11 (1949).
25. James, N. Bacterial Contamination from the Homogenizer—Its Control. Thesis. Ia. State Coll. 1923.
26. *Milk Dealer*, **28** (4), 42 (1939).
27. Trout, G. M. *Homogenized Milk*, 181, 1950. Michigan State College Press, East Lansing.
28. *J. Milk Technol.*, **1**, 20 (1938).
29. *U. S. Public Health Marine Hospital Service Hyg. Lab. Bull.* 56, 683 (1909).
30. *J. Exptl. Med.*, **4**, 217 (1899).
31. *Wis. Agr. Expt. Sta. 17th Ann. Rept.*, 147 (1900).
32. *U. S. Public Health Marine Hospital Service Hyg. Lab. Bull.* 42 (1908).
33. *J. Am. Vet. Med. Assoc.*, **49**, 678 (1916).
34. *Am. Rev. Tuberc.*, **15**, 399 (1927).
35. *Brit. Med. J.*, **2**, 1080 (1933).
36. *Can. Public Health J.*, **25**, 13 (1934).
37. *Am. J. Public Health*, **13**, 807 (1923).
38. *Lancet*, **205**, 317 (1923).
39. *Public Health*, **38**, 297 (1925).
40. *J. Hyg.*, **25**, 273 (1926).
41. *Med. Officer*, **61**, 177 (1939).
42. *Lancet*, **210**, 223 (1926).
43. *J. Hyg.*, **26**, 392 (1927).
44. *Am. J. Public Health*, **20**, 615 (1930).
45. *Am. J. Public Health*, **43**, 185 (1953).
46. *J. Bacteriol.*, **2**, 185 (1917).
47. *J. Bacteriol.*, **15**, 41 (1928).
48. *Am. J. Public Health*, **18**, 710 (1928).
49. *J. Bacteriol.*, **21**, 54 (1931).
50. *J. Dairy Sci.*, **15**, 6 (1932).
51. *J. Bacteriol.*, **24**, 461 (1932).
52. *J. Am. Med. Assoc.*, **68**, 1305 (1917).
53. *J. Infectious Diseases*, **29**, 310 (1921).
54. *U. S. Public Health Serv. Public Health Bull.* 147 (1925).

55. *Am. J. Public Health,* **42,** 525 (1952).
56. *Public Health Repts.,* **64,** 499 (1949).
57. *Am. J. Hyg.,* **55,** 246 (1952).
58. *J. Milk and Food Technol.,* **16,** 263 (1953).
59. *Wis. Agr. Expt. Sta. 18th Ann. Rept.,* 185 (1901).
60. *U. S. Dept. Agr. Tech. Bull.* 18 (1927).
61. *Proc. Soc. Appl. Bacteriol.,* **13** (1), 1 (1950).
62. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 161 (1913).
63. *Public Health Repts.,* **64,** 1411 (1949).
64. *Mich. Agr. Expt. Sta. Quart. Bull.,* **30,** 64 (1947).
65. *J. Dairy Sci.,* **32,** 29 (1949).
66. *Proc. Intern. Assoc. Milk Dealers 31st yr,* 179 (1939).
67. *J. Dairy Research,* **6,** 125 (1935).
68. *J. Bacteriol.,* **8,** 127 (1923).
69. *Vt. Agr. Expt. Sta. Bull.* 275 (1927).
70. *J. Bacteriol.,* **18,** 333 (1929).
71. *J. Dairy Sci.,* **13,** 273 (1930).
72. *J. Dairy Sci.,* **12,** 385 (1929).
73. *J. Dairy Sci.,* **14,** 27 (1931).
74. *J. Bacteriol.,* **36,** 83 (1938).
75. *J. Dairy Research,* **7,** 182 (1936).
76. *B. W. Hammer Panegyric,* 177 (1937). Collegiate Press, Inc., Ames, Ia.
77. *J. Dairy Research,* **11,** 274 (1940).
78. *J. Milk Technol.,* **6,** 17 (1943).
79. *J. Bacteriol.,* **9,** 285 (1924).
80. *J. Bacteriol.,* **15,** 179 (1928).
81. *J. Milk Technol.,* **3,** 165 (1940).
82. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 196 (1932).
83. *Dairy Inds.,* **14,** 586 (1949).
84. *J. Dairy Sci.,* **11,** 18 (1928).
85. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 134 (1928).
86. *Neth. Milk Dairy J.,* **5,** 75 (1951).
87. *J. Agr. Research,* **2,** 321 (1914).
88. *J. Bacteriol.,* **22,** 275 (1931).
89. *Appl. Microbiol.,* **1,** 47 (1953).
90. *J. Agr. Research,* **2,** 167 (1914).
91. *J. Milk Technol.,* **6,** 269 (1943).
92. *N. Y. State Assoc. Dairy and Milk Inspectors,* 13, 195 (1939).
93. *Milk Plant Monthly,* **35** (8), 30 (1946).
94. *Assoc. Bull. Milk Ind. Foundation,* **39,** 195 (1947).
95. *J. Soc. Dairy Technol.,* **4,** 177 (1951).
96. *21st Ann. Rept., N. Y. Assoc. Milk Sanitarians,* 53 (1947).
97. *Dairy Inds.,* **13,** 135 (1948).
98. *Intern. Assoc. Dairy and Milk Inspectors 12th Ann. Rept.,* 204 (1933).
99. *Ia. Agr. Expt. Sta. Bull.* 286 (1931).
100. *J. Soc. Dairy Technol.,* **4,** 245 (1951).
101. *J. Milk Technol.,* **4,** 195 (1941).
102. *J. Milk Technol.,* **7,** 251 (1944).
103. *J. Milk Technol.,* **5,** 269 (1942).
104. *Intern. Assoc. Milk Dealers 34th yr,* 127 (1941).
105. *J. Dairy Sci.,* **23,** 502 (1940).
106. *J. Soc. Dairy Technol.,* **4,** 51 (1950).
107. *Proc. Soc. Appl. Bacteriol.,* 51 (1946).
108. *J. Agr. Research,* **3,** 401 (1915).
109. *J. Dairy Sci.,* **12,** 202 (1929).
110. *J. Dairy Sci.,* **13,** 94 (1930).
111. *J. Dairy Sci.,* **19,** 495 (1936).
112. *J. Milk Technol.,* **7,** 20 (1944).
113. *J. Milk Technol.,* **9,** 21 (1946).
114. *Svenska Mejeritidn.,* **46,** 111 (1954).
115. *J. Milk and Food Technol.,* **10,** 137 (1947).
116. *J. Dairy Sci.,* **29,** 651 (1946).
117. *Milk Plant Monthly,* **22** (2), 26 (1933).
118. *J. Dairy Sci.,* **24,** 305 (1941).
119. *J. Milk Technol.,* **4,** 128 (1941).
120. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 754 (1941).

121. *Dairy Sci. Abstr.*, **7**, 52 (1945).
122. *J. Dairy Research*, **17**, 261 (1950).
123. *J. Dairy Sci.*, **21**, 219 (1938).
124. *J. Dairy Sci.*, **24**, 526 (1941).
125. *Refrig. Eng.*, **44**, 370 (1942).
126. *W. Va. Agr. Expt. Sta. Bull.* 255 (1933).
127. *Nations' Health*, **5**, 1 (1923).
128. *Z. Hyg. Infektionskrankh.*, **17**, 272 (1894).
129. *Public Health Repts.*, **47**, 1951 (1932).
130. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 166 (1913).
131. *Milk Plant Monthly*, **30** (7), 26 (1941).
132. *J. Dairy Sci.*, **23**, 1131 (1940).
133. *J. Dairy Sci.*, **28**, 29 (1945).
134. *J. Nutrition*, **26**, 337 (1943).
135. *J. Am. Dietet. Assoc.*, **20**, 226 (1944).
136. *J. Dairy Sci.*, **28**, 259 (1945).
137. *J. Dairy Sci.*, **28**, 269 (1945).
138. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 254 (1940).
139. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 261 (1941).
140. *J. Dairy Sci.*, **36**, 356 (1953).
141. *Milk Ind. Foundation Conv. Proc.*, **4**, 5 (1953).
142. *Public Health Repts.*, **68**, 769 (1953).
143. *Med. Research Com. Spec. Rept.* 49 (1920).
144. *J. Hyg.*, **24**, 123 (1925).
145. *J. Dairy Sci.*, **2**, 374 (1919).
146. *Am. J. Public Health*, **17**, 221 (1927).
147. *J. Dairy Sci.*, **13**, 368 (1930).
148. *Science*, **76**, 391 (1932).
149. *Am. J. Public Health*, **4**, 224 (1914).
150. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **40**, 109 (1914).
151. *Intern. Assoc. Dairy and Milk Inspectors, 24th Ann. Rept.*, 69 (1935).
152. *Milchwissenschaft*, **6**, 315 (1951).
153. *J. Dairy Sci.*, **24**, 1055 (1941).
154. *Food Inds.*, **19**, 1495 (1947).
155. *Proc. Soc. Appl. Bacteriol.*, **16** (1), iv (1953).
156. *Intern. Dairy Congr., Proc. 13th Congr.*, **2**, 703 (1953).

Milk Plant Operation

8

Operation of modern milk plants has been developed to the point where satisfactory products can be marketed constantly. Suitable grading of the incoming milk and proper handling result in good flavor. Adequate pasteurization and control of recontamination ensure products that are free of disease-producing organisms and keep well. Best results in plant operation require attention to many details, and carelessness may result in products that bring complaints and cancellation of orders.

Selection of Milk. Each shipment of milk used must meet certain standards that vary widely in different areas and different plants. A minimum standard requires that all the milk have a satisfactory flavor and appearance. Commonly, to this are added other requirements set by regulatory officials or plant management, such as a bacterial standard, condition of the cows and farm equipment, amount of sediment, delivery temperature, etc.

The principal off flavors that cause rejection of milk are those due to bacterial action and certain feeds. The former are controlled readily by proper farm and delivery methods, and the latter are at least improved by better feeding procedures although feed flavors sometimes cause serious problems. Abnormal appearance of milk may be due to bacterial action, udder abnormalities, etc.

Handling Raw Milk. The raw milk accepted for processing requires proper handling. Contaminated dump tanks, pumps, pipe lines, storage tanks, etc., increase the bacterial count of the raw milk; storage tanks frequently are poorly cleaned and sanitized. If milk is to be held raw for a time, it should be cooled, unless already sufficiently cold, to prevent bacterial growth. Since the raw milk is used to partly cool the pasteurized milk in any regenerative system, the refrigeration needed for the raw milk is largely reclaimed. Filtration or clarification is used to remove most of the small amount of sediment permitted by receiving platform standards.

Pasteurization of Milk. Pasteurization, whether HTST or LTLT, should be carried out in properly cleaned equipment with adequate attention to the temperature and time of heating. Recording thermometers, suitably checked, are a necessity. With HTST heating, the flow diversion valve must be operating properly. Frequent phosphatase tests can be used to confirm adequate heating. Serious overheating can be recognized by the flavor of the final product and, with unhomogenized milk, by the cream line. The pasteurized milk must be properly cooled before bottling, at least to 38 F and preferably to 35 F.

CONTAMINATION SUBSEQUENT TO PASTEURIZATION

In a well-operated plant, contamination incident to bottling the pasteurized milk is negligible but, with inadequate equipment sanitation, etc., it is very significant. With contaminated equipment, the organisms added per milliliter of milk decrease as the equipment is used, often very rapidly, so the first material through the equipment should be studied in considering contamination from this source. The closed lines from the raw milk tank to the pasteurizer and bottle filler, now often used, may complicate the sampling in studying portions of the equipment but, if counts on the freshly bottled milk are unsatisfactory and heat resistant organisms in the raw milk are not involved, the equipment needs attention. Successive bottles from a filler may show very different bacterial counts due to contamination from certain valves. Rubber parts can be treated readily by holding them in 0.5% lye solution.

General Equipment. Any of the equipment with which milk comes in contact may be a source of organisms. Lines, pumps, vats, coolers, fillers, etc., all have been found responsible for serious contamination of milk. The possibilities along this line are shown by some of the older data.

Judkins and Downs,[1] with equipment which was rinsed with scalding water before the processing operation began, noted that in 6 trials the pasteurized milk in the vat averaged 439.5 bacteria per ml; at the bottler it averaged 12,195; and in the bottles it averaged 48,033. Accordingly, the pump and piping taking the milk to the bottler added 11,755.5 bacteria per ml, and the bottling process added 35,838 more. Smith [2] carried out 8 tests at a large milk plant and found the average count on the cooled milk was 2,038 per ml, whereas on the bottled milk it was 5,744; since the bottles were practically sterile, the organisms must have come largely from the bottle filler. In another investigation [3] the average bacterial content of the milk from the cooler was 8,452 per ml, whereas that of the bottled milk was 15,592.

When a bottle filler which had stood for 20 hr after washing was not steamed before use, Prucha *et al.*[4] noted a conspicuous increase in the bacterial content of the bottled milk; in 10 trials in which the maximum count for the milk from the pasteurizer was 203 and the average 84 per ml, the maximum for the first bottle through the filler in each trial was 209,600 and the average 96,900.

The negligible contamination incident to bottling milk with adequately treated equipment is evident from the following counts obtained at Purdue University on milk from 3 plants.

Negligible Contamination Incident to Bottling Milk with Adequately Treated Equipment

Sample Number	Plate Count per Milliliter			Coliforms per Milliliter in Bottled Milk
	From Pasteurizer	At Bottler	In Bottle	
1	1,100	1,800	1,900	<1
2	700	790	820	<1
3	15,000	16,000	16,000	2
4	6,300	6,600	6,900	<1
5	2,600	2,800	2,800	<1
6	7,900	9,100	9,700	6
7	2,500	2,700	2,700	1
8	18,000	19,000	19,000	2

Glass bottles. In some instances glass bottles have been sources of significant numbers of bacteria. Smith [2] studied 268 bottles, mainly pints, and found that from <1 to 1,636 bacteria would have been added per ml of milk. Later,[3] 344 bottles from 32 plants were studied. Without a mechanical washer and steamer, contamination from 190 bottles would have averaged 458.2 per ml, with a range from <1

to 9,005; with a mechanical washer and steamer, contamination from 154 bottles would have averaged 78.8, with a range from <1 to 900.

In Great Britain, Hobbs and Wilson [5] examined 2,406 bottles from 105 bottle-washing plants of 26 types. The mean counts, reckoned as pints, at 71.6 and 98.6 F (22 and 37 C), respectively, of all bottles washed in large plants were 2,008 and 2,324; in small rotary plants, 7,982 and 7,389; in plants using steam sterilization, 23,671 and 9,704; and, in plants with hand washing only, 96,065 and 37,818. Taking all large plants together, 55.5% of the bottles had less than 600 colonies at 71.6 or 98.6 F, and only 1.1% had over 25,000. The authors noted that virtual sterility can be achieved either by using relatively strong caustic soda solutions at low temperatures or relatively weak solutions at high temperatures.

Because of carelessness on the part of some milk users, the glass bottles returned to plants vary widely in physical appearance and microbiological condition. This is shown by the following data, obtained by Bryan *et al.*[6] on quart bottles returned to a plant.

Condition of Bottles Returned to a Plant

Bottle Number	Physical Condition	Bacteria Present
1	Milky sediment	80,000,000
2	Brown dirt (dry)	30,000,000
3	Milky film	850,000
4	Fairly clean	12,000
5	Clean	300
6	Clean	20

With proper maintenance and operation, modern bottle washers deliver bottles that are clean and contain very few organisms, even when many of the bottles fed into them are dirty.

Requirements covering milk bottle washing commonly include alkalinity and temperature of the washing solution and period of exposure. A final rinse with a bactericidal solution ensures a satisfactory microbiological condition under normal operation; it has been found to reduce satisfactorily the bacterial content of bottles that come to it with large numbers of bacteria.[6] Stark *et al.*[7] found tetrasodium pyrophosphate in the washing solution essential in obtaining satisfactorily rinsed bottles; temperatures of 170 F, or higher, were not needed to maintain low bacterial counts on bottles, and at 160 F bottles had the best physical appearance.

Colony estimates of 1,000, 500, and 250 per quart, pint, and half-

pint bottles are considered satisfactory,[7] but properly treated bottles commonly show much lower counts and sometimes no detectable organisms. At Purdue University, counts on bottles from a washer ranged from 2 to 18 for half-pint bottles (4) and from 8 to 32 for quart bottles (8).

Bottle Caps. Bottle caps constitute a possible source of milk contamination and, when exposed to flies, dust, etc., may be of importance. Regulations commonly require adequate protection of bottle caps. Growth of organisms does not occur on caps that are properly stored, and the organisms present on caps are added directly.

Smith [2] found in the examination of 2,166 bottle caps, taken in lots of 28 to 68 from the supplies in use at different pasteurization plants, that if the caps had been used on bottles of milk they would have added from <1 to 453 organisms per ml of milk. The method of examination used was to wash a number of caps in sterile water, determine the bacteria in the water, and then calculate to one-half the surface of a cap.

As caps are handled in modern capping equipment, they are of no significance as a source of organisms in milk. However, when carelessly handled they may be of importance. Capping bottles by hand is very objectionable from the bacteriological standpoint and is prohibited by various regulations.

Paper Bottles. Following extensive study of the paperboard used in their manufacture, methods of moisture proofing, etc., paper bottles were accepted as satisfactory containers for milk. Many milk purchasers prefer them to glass because they are light, store easily in a refrigerator, and are so readily disposed of. A large percentage of the fluid dairy products now going to consumers is supplied in paper.

Normally, paper bottles contain very few organisms and adequately protect milk against contamination. Tanner [9] emphasized that careful methods of manufacture result in a paper container with a very low bacterial count and that pathogens cannot survive the various procedures involved. Paperboard used for milk containers, milk bottle caps, etc., can be examined for numbers of bacteria by disintegrating and plating.[8] Standards for the material have been established. The finished containers can be examined by a rinse procedure, sterility test, etc.

Wheaton *et al.*[10] found with 4,933 paraffined containers that about 80% yielded no colonies on agar plates, whereas over 90% of the remainder showed fewer than 5 colonies per container, the great majority showing but 1 colony. Mudge and Foord,[11] using the needle

puncture method, noted that about 95% of the paraffined half-pint containers were sterile, as were a somewhat smaller percentage of the quart containers. In trials by Rice,[12] broth sterility tests made on a total of 1,600 paper containers, selected uniformly from several moisture-proofing treatments, indicated that approximately 80% were sterile. Prucha and Tracy[13] found the paper milk containers on the market were in excellent sanitary condition. Of 2,607 quart containers, fabricated at several carton factories from paperboard manufactured at different mills, 878 (34%) were sterile, and those not completely freed of bacteria contained, as a rule, only a few; tests for coliform organisms on many of the containers were negative. Bacteria did not penetrate the walls or seams of paper containers. After filled containers had been submerged in a bacterial suspension for as long as 48 hr, none of the bacteria from the suspension were in the milk. When containers were heavily contaminated and then paraffined, only an occasional container showed the presence of the inoculated organism. With containers not packed in cases, the temperature of the milk in paper changed a little more slowly than in glass; when packed in cases that were completely filled, the temperature change definitely was slower in the paper. All the paper bottles tested stood as much as 80 lb pressure before they leaked. The authors noted that paperboard commonly contains some bacteria, mostly in the spore stage, which cannot be destroyed by the heat of the paraffin. The practically sterile condition of the containers indicates that the paraffin imprisons most of the organisms and keeps them from getting into the milk.

In studying the bactericidal effect of the paraffining of paperboard used for paper milk containers, Moss *et al.*[14] used a culture of *E. coli* having a thermal resistance such that a skim milk culture in sterile skim milk showed a 99% reduction at 140 F for 20 min. Under the test procedure followed, in which moisture conditions of the paperboard were controlled, 99% reduction of the test organism due to paraffining was obtained in about 10 sec at 180 F; 20 sec at 175 F; 35 sec at 170 F; 2 min at 165 F; and 4 min at 160 F. The authors suggested that, if use of relatively high paraffin temperatures is impracticable, it may be necessary to subject the containers to separate bactericidal and waterproofing treatments or provide means for increasing the bactericidal effect of the paraffining. Relatively low paraffin temperatures sometimes are desired to reduce excessive drain-off before cooling which may leave fibers unprotected.

Plant Employees. Employees in plants handling milk may add organisms to it. Because the contamination of milk from human sources may include pathogens, it is especially serious when it occurs subsequent to pasteurization since this process so regularly is assumed to provide definite protection. Modern milk equipment, which almost entirely eliminates direct human contact with the milk after pasteurization, constitutes an important advance from the sanitary standpoint.

Sweet Water Cooling Systems. The circulation of refrigerated water, instead of brine, for the cooling of dairy products sometimes is practiced in plants. Advantages of the systems are (1) smaller refrigeration units may be practical, (2) lower operating costs when an off-peak electric rate is available, and (3) the products cooled are never frozen.

In some cases sweet water systems have been responsible for defects in the products cooled. Bacteria may increase in the water until there are large numbers present and then, due to some irregularity, especially a leak, the product may be contaminated with them. Because of the temperature of the cooling water, the organisms are essentially psychrophilic types which can multiply at the temperatures used for holding milk and cream.

CARE OF PLANT EQUIPMENT

All equipment with which milk comes in contact requires proper washing and sanitizing. For many years this was essentially a hand operation but, recently, cleaning by circulating solutions through the equipment (circulation cleaning) has been perfected.

Hand Cleaning. With a hand operation, the cleaning of equipment involves rinsing with cold or warm water, scrubbing with hot water containing washing reagent, and then careful rinsing with hot water; if considerable fat is present, as when the equipment has been used for cream, it is essential to use warm water for the initial rinse. Scrubbing is a step in the process that often is poorly done. In pasteurizing equipment, in the upper parts of coolers, and wherever milk or cream is exposed to high temperatures, there is more than the usual tendency for the milk solids to stick to the metal, and without thorough scrubbing the cleaning will be incomplete. If careless treatment continues, there is a gradual accumulation of material that interferes with the heat transfer, develops objectionable flavors, and may permit growth of organisms. The scrubbing should not be limited to surfaces that are easily reached but should include every part of the equipment.

In the cleaning of equipment that is complicated in construction, disassembling for washing should be as complete as possible.

The washed equipment is heat treated at once with hot water or steam. The heating should be thorough so that, with complete drainage, the remaining moisture will evaporate. In some plants the heating is so poorly done that the expected results cannot be obtained. Exposed surfaces require special attention, if the heating is to be adequate. It is not the presence of hot water or steam but the heat supplied by them that accomplishes the destruction of organisms. The temperature of the equipment is raised to the desired point only when the proper time factor is provided. Sanitizing equipment with a bactericidal solution just before use is practiced widely.

Circulation Cleaning. With circulation cleaning (CC) or cleaning in place (CIP), the equipment is (1) rinsed with water, (2) washed by pumping through it one or more hot cleaning solutions at considerable velocity for a considerable period (a line returns the solution to the tank so it can be reheated and its use continued), (3) again rinsed, and (4) finally sanitized with a bactericidal solution and/or hot water.

By lowering labor costs, CC is an important step in reducing the cost of operating a plant. Also, it makes for a better operation since hand cleaning is the most unpleasant task in a dairy plant and one that often is slighted. In general, CC results in less damage to pipes, fittings, etc. Other suggested advantages [15] are more uniformly satisfactory bacteriological results, detergents are not limited to those nonirritating to the skin, and pipes can be mounted higher to give additional head room. CC was first applied to pipe lines but has been adapted to other equipment.

Pipe Lines. Dismantling, cleaning, and reassembling of pipe lines is very time-consuming. Lines satisfactory for CC have been classified as follows: [16] (1) Stainless steel or glass with gaskets; the gaskets should be of low absorbent, relatively stable material which is smooth and nontoxic and should be self-positioning and of such design as to form a substantially flush interior joint. (2) Stainless steel with fittings having no gaskets; the fittings should have self-positioning joints of such design and finish as to form a smooth, flush interior joint. (3) Stainless steel or glass with welded connections; the interior surface of any weld should be flush and drainable and have a finish equivalent to adjacent surfaces.

Following earlier considerations of the problem, Moore *et al.*[17] studied a permanent sanitary pipe line, consisting of four polishes of

stainless steel and pyrex glass, to see if it could be cleaned satisfactorily by rapid circulation of acid and alkaline detergents and sanitized by circulating water at 180 F. Milk samples from the inlet and outlet of the line were examined, sections of the tubing were swabbed regularly, and visual inspections of the line were made weekly. There was no measurable pick-up of organisms from the permanent line. Swab counts on the line were low. Under the conditions of the study, a permanently installed pipe line of either stainless steel or glass was satisfactory from the sanitary standpoint.

Additional investigations have shown that, with the proper procedure, CC of lines is satisfactory from the bacteriological standpoint. Moore [18] reported that with CC of lines, the maximum plate count on the pasteurized milk over a 15-day period was 520 per ml for the beginning of the run and 1,020 for the end of the run, while corresponding minimum counts were 25 and 30; all coliform counts were <1 per ml.

Parker *et al.*[15] noted that the bacteriological condition of CIP lines remained excellent at all times, regardless of the cleaner used, and was independent of the physical condition of the lines. In some instances swabs were taken from areas of definite fat films; the highly alkaline detergents and the high temperatures permitted by CC appeared to assure a low bacterial count. The only coliform organisms detected on line swabs during the entire 12-month study were noted after use of the conventional "take down and brush" procedure.

The methods used for CC of lines in different studies vary somewhat, but mainly in detail, and all rely primarily on circulating strong reagents at considerable velocities through lines with substantially flush interior joints. Both acid and alkaline cleaners often have been used in an operation and the order of use has varied. Parker *et al.*[15] reported that, with the soft water conditions encountered, acid cleaner was not needed. Various temperatures have been used with the cleaning solutions but relatively high temperatures (160 to 170 F) appear most satisfactory. Commonly, a cleaner is circulated for a rather extended period (30 to 40 min). Moore [18] reported that when acid and alkaline solutions were circulated for 20 instead of 40 min milk stone began to accumulate and pin-point colonies were noted on plates. The circulation pump should give considerable velocity for effective cleaning. Manufacturers of cleaning reagents can give advice on the cleaners to use and their concentrations under various water and other conditions.

Valves in a line commonly receive special attention. The plugs are removed and both the plugs and seats brushed with a detergent, rinsed, sanitized, and the plugs replaced. Such treatment has been given either before or after circulating a detergent. Caps on lines, etc., also may require special treatment.

HTST Pasteurizers. CC is used effectively with HTST pasteurizers.[19, 20] Often the pasteurizer is combined with certain lines and perhaps other equipment, the flow diversion valve being disconnected. After treatment the pasteurizer should be opened and perhaps the plates brushed and rinsed with water. Critical parts [19] appear to be the heating and regenerative sections, but ordinarily any material left here is easily removed with light brushing.

Moore and Potter [19] reported the following plate and coliform counts on homogenized, pasteurized, bottled milk processed in a CIP plate type pasteurizer.

Counts on Milk Processed in CIP Plate Type Pasteurizer

Bacteria per Milliliter	Number of Counts in 84 Operating Days			
	Plate Count		Coliform Count	
	First of Run	End of Run	First of Run	End of Run
<1	0	0	64	60
<10	0	0	76	79
11 to 20	0	0	5	4
21 to 30	0	0	3	1
100 to 499	43	48	0	0
500 to 999	32	31	0	0
1,000 to 3,000	7	5	0	0
>3,000	2 *	0	0	0

* 3,760 and 3,800.

Additional Equipment. CC also is being used for additional equipment. Storage tanks are being cleaned by installing a perforated pipe so that the circulated materials can be forced over the entire inside of the tank. Also, a pipe with a perforated bulb at the end can be moved slowly about in the tank by hand so that the circulated materials effect adequate cleaning. Separator discs and small equipment parts are cleaned by circulating materials around them in a tank.

Control Procedures. Results obtained in a plant with CC may be influenced by the way certain equipment is operated, by the nature

of the water supply, etc., so that when the procedure is introduced it should be checked by frequent visual examination of the lines and by bacteriological tests, involving both plate counts on milk and swab tests on lines, valves, etc., until its efficiency has been established. Holland *et al.*[20] noted that, when a HTST unit was used for chocolate milk, the cleaning problem was much more difficult. Doubling concentrations of both acid and alkali used for ordinary cleaning removed material deposited by the product.

GENERAL CONSIDERATIONS

Cans Returned to Farms. When milk is received in cans, those returned to the farms need constant attention. They should be clean, dry, free from objectionable odors, and low in bacteria (1,000 per can and cover are satisfactory, regardless of size[8]); if not in good repair they should be reconditioned or discarded. The can washer requires proper maintenance and cleaning and proper operation from the standpoints of washing compound strength, temperatures, etc. Many farms use returned cans with no additional treatment and, if cans are received in poor condition, it suggests that the milk production methods also can be lax.

Tracy[21] found in a survey of 13 plants that many of the cans were unsatisfactory from the bacteriological standpoint. Individual cans from a washer varied considerably, and no washer yielded sterile cans. Frequently, new cans with shiny surfaces gave high counts. Cans with open seams consistently showed such counts. Some cans washed with acid cleaners gave an alkaline reaction. Of all the cans studied, 51.7% were free of rust, milkstone, pitting, corrosion, and deposits of organic or inorganic matter, and of these 56.57% were satisfactory from the bacteriological standpoint; of the cans in poor physical condition, 47.18% were satisfactory. The heat received by a can during washing was the important factor determining bacterial destruction. Temperatures above 160 F commonly gave satisfactory results. Chlorination of cans at the time of washing reduced the bacterial counts on freshly washed cans, but the bacterial populations of such cans increased when held for 48 hr at room temperature and approximated those of freshly washed, unchlorinated cans. The wash water and air blast were found to be possible sources of contamination.

Clean cans, low in bacteria, are a necessity when cans are used in milk delivery, and there should be frequent inspection of the cans going back to farms. Wiping the inside of a can with a clean cloth

or paper towel simplifies the detection of foreign material and traces of moisture. Some plants have found that adequate maintenance of a washer requires frequent inspections by someone thoroughly familiar with the construction of the equipment.

Storage of Milk in Bottles or Cans. Milk that is to be bottled or canned for delivery should be well cooled, certainly to 38 F and preferably a few degrees lower. As soon as the bottles or cans are filled they should go immediately to a refrigerated room or truck where the temperature is at least as low as that of the milk. Adequate temperatures for cooling and holding the properly processed milk ensure control of bacterial growth in the product.

Some plants have more difficulty with the keeping quality of market cream than with milk. Sometimes this is due to inadequate cooling of the cream before bottling.

Distribution of Bacteria in Bottled Milk. With homogenized milk, in which there is no significant fat separation on holding, distribution of organisms in the product remains rather uniform, but with unhomogenized milk the rising fat carries many bacteria with it. Torrey and Rahe [22] found that the upper 2 oz of the cream of fresh bottled milk of fair quality contained, on the average, 50 to 100% more bacteria than an equal amount of the lower cream, whereas with older and more grossly contaminated milk the lower cream might contain as many, or even more, bacteria than the upper layers; a higher percentage of bacteria was brought to the surface layers in milk rich in fat than in milk poor in fat. After being held 1 hr in bottles the milk showed no distinct cream layer and only a slight increase in bacteria at the surface; it was not until a cream layer began to form that an excess of bacteria in the upper layers was noted.

Stine [23] found that when quart bottles of milk were held at 32 F for 6 or 30 hr the cream had much higher bacterial counts than the milk beneath. Counts on the top of a cream layer were higher than those on the lower portions, and the counts appeared to decrease progressively at greater depths in a bottle. Added yeasts showed a greater tendency to accumulate at the top of a bottle of milk than did bacteria.

Nicholas *et al.*[24] bottled and capped fresh milk in 12 clean quart bottles, with precautions against contamination, and suspended them in the water bath of an electric milk cooler. The water came nearly to the tops of the bottles and cooling was accelerated by agitating it. At 15-min intervals 40 ml of material was removed from the top of a bottle for plating and fat determination, and the temperature of the milk was noted. The cooling water remained between 42 and 43 F

and the milk dropped rapidly to below 50 F. Under the conditions the increase in fat at the top of a bottle was soon significant. Bacterial filtration accompanied the fat separation, resulting in a concentration of organisms in the cream layer that finally reached 97 to 99% of the total count. Rate of separation and filtration was maximum between 1 and 2 hr and practically complete in 2 hr.

Figure 26. Refrigeration system for loaded milk trucks.

Bacterial Growth in Pasteurized Milk. Properly pasteurized milk that has been bottled without significant contamination has excellent keeping qualities at refrigerator temperatures. This permits less than daily delivery of milk to homes and markets and also the holding of bottles in refrigerators for extended periods. The period pasteurized milk will keep depends on various factors, such as the refrigerator temperature, quality of the raw milk, and care used in processing. When spoilage occurs in refrigerated milk, it is due to psychrophiles. *Ps. fragi* is especially important in this connection, as is evident from the odor of spoiled refrigerated milk in various parts of the United States. Burgwald and Josephson [25] noted stale, unclean, acid, and bitter flavors in stored pasteurized milk and also that psychrophiles apparently are responsible for acid development in milk at 40 F.

Normally, psychrophiles are destroyed by pasteurization (see p.

187). Rogick and Burgwald[26] never found psychrophiles in 4.1 ml of milk pasteurized with either the LTLT or HTST process. The effect of pasteurization on psychrophiles is shown by the following data obtained at Purdue University on milk held at 40 F; the bacterial count on the raw milk increased greatly during holding, but the count after pasteurization changed very little.

Bacteria per Milliliter, before and after Pasteurization, in Milk Held at 40 F

Age of Milk	Bacteria per Milliliter	
	When Raw	After Pasteurization
Fresh	16,200	200
24 hr	23,000	240
48 hr	560,000	300
72 hr	890,000	310
96 hr	1,100,000	340

Pasteurized milk may be contaminated with psychrophiles from equipment with which it comes in contact and from glass bottles.[26,27] Since psychrophiles constantly are being brought into a plant by raw milk, water, soil, returned bottles, etc., there often is an opportunity for their distribution about the plant.

With commercially pasteurized homogenized milk, Boyd *et al.*[28] found that when the storage temperature was lowered from 40 to 33 F the keeping quality was extended 11 to 14 days. In all samples flavor deterioration was closely associated with growth of psychrophiles. The keeping quality of samples collected in the fall and winter was poorer than of those collected in summer; the difference was greater at 33 F than at 40 F.

Keeping Quality Tests. Keeping quality tests, in which the bottled products of a plant are held under normal consumer conditions and examined for off odors, flavors, etc., after selected holding periods, are helpful in establishing that the products maintain their quality satisfactorily. Such tests often detect minor irregularities in the processing before they become serious and also are useful in answering unjustified criticisms of the products. Olson *et al.*[27] noted that standard plate and coliform counts on freshly pasteurized milk, as routinely performed, are not good indicators of keeping quality.

Consumer Complaints. When an appreciable number of consumers complain that the milk rapidly sours or develops other defects, a check

on the quality of the milk received and on all steps in its handling, holding, and delivery is indicated. This requires repeated bacterial counts and observations on temperatures, etc., since irregularities may occur only occasionally.

While the plant operation is being checked, the conditions in stores selling the milk should be considered. Perhaps the holding temperature there is too high or the bottles are not properly rotated. Irregularities after the milk reaches the homes also are a possibility, especially when complaints are few. The refrigerator may not be operating properly or the milk may be held at room temperature for long periods. Also, the bottled milk may be poured into utensils; this often results in significant contamination and, moreover, permits absorption of odors. Rapid development of defects in market cream requires the same study that is used for milk. In this connection use of the poorer quality or older milk for separation may be a factor.

Bulk Milk Dispensing. The bulk dispensing method of serving milk in food establishments, which is being extended in certain areas, requires special attention by milk processors in preparing the cans and attaching the rubber tubes and in filling and sealing the cans. Persons using the dispensers may need instructions in temperature maintenance and in avoiding contamination of the milk.

Noles and Wilkowske [29] found that on 4 days, covering a 2-week period, one food establishment dispensed milk with plate counts from 3,000 to 4,200 per ml and coliform counts of <1 while another establishment, using poor sanitary practices, dispensed milk with plate counts from 6,300 to 68,000 per ml and coliform counts from <1 to 200; on each day the two establishments were using milk from the same processor and the cans had the same dates.

SUPERVISION OF MARKET MILK SUPPLIES

As with many food products, market milk supplies require supervision. Early efforts along this line involved primarily chemical composition (to control watering and skimming) and freedom from preservatives. When the role of milk in the spread of diseases was clearly recognized, attention was directed to the safety of the product (absence of pathogenic bacteria) and to cleanliness in production and handling.

Public Health Service Ordinance and Code. The federal government long has been concerned with the safety of milk and milk products. The Office of Milk Investigations was established in 1923.

When studies of milk regulations in force in 1923 revealed that regulations varied greatly, with some of little or no public health significance and others containing impractical requirements, a standard milk ordinance was published in 1924. An accompanying code, to provide for uniform interpretation of the ordinance, was published in 1927. Since then the ordinance and code has been revised as needed to keep abreast of developments in public health, dairy science, veterinary medicine, and similar fields. It includes the ideas of persons in federal, state, local, and educational agencies and of milk sanitarians and the dairy industry itself. Thus it represents the thought of the people best qualified to prepare a milk ordinance. The ordinance and code is used to regulate the milk served on interstate carriers and is incorporated in federal specifications. It also has been adopted widely by municipalities and counties, serves as the basis for laws in many states and territories, and has been upheld by court decisions.

The ordinance and code covers in detail all points of significance in satisfactory milk production, processing, and handling; on certain points optional choices are offered. It is the authority on the best procedures along these lines, and anyone interested in adequate milk supplies should so consider it and use it in deciding points of controversy. It provides for various grades of milk, when these seem necessary or desirable, although in general the more grades sold in an area the more complicated is the supervision. Also, it recommends that every effort be made to limit the sale of milk to a pasteurized product.

Local Supervision. A city or county department of health commonly is responsible for regulation of milk supplies at the local level. Efficient local supervision has resulted in many excellent milk supplies because of a special interest in milk production and distribution and a detailed understanding of conditions in the area. Through good leadership a supply can be improved until it is above any level likely to be attained over an extended area. In an emergency, action can be taken quickly.

State Supervision. Enforcement of laws enacted by a state usually is the responsibility of the department of health or department of agriculture. Supervision of milk supplies in a particular area commonly is done with the aid of the local agency. State control provides some protection for localities that otherwise might not have it because of lack of interest. It can exclude from regular supplies milk of inferior quality, instead of permitting it to be sold in areas other than the one in which it was found unsatisfactory.

Industry Supervision. Dairy plants commonly supervise the production and handling of milk by their patrons to make certain that it meets the requirements of regulatory agencies and also their own requirements. Many larger plants maintain a laboratory and/or a field service to aid patrons maintain proper control over their methods. A laboratory, with plant and product inspections, also aids in the proper control of plant procedures. Small dairy plants often obtain bacterial counts and other types of examinations through a commercial laboratory. Adequate records of bacterial counts, inspections, etc., when frequently reviewed, give the plant management a broad picture of the operations that cannot be obtained in any other way.

Need for Uniform Regulations. Even with the wide adoption of the U. S. Public Health Service Milk Ordinance and Code, variations still exist in milk regulations in the United States, as is evident from a recent survey.[30] Uniform regulations are highly desirable and lack of uniformity throughout a producing area, because the milk happens to go to different cities, may lead to complications. In some producing areas milk from adjoining farms may go to different cities. Without uniform regulations, the city with the less rigid regulations may have the simpler procurement problem. Apparently, milk producers sometimes have changed their markets in order to operate under less rigid requirements. Milk distributors selling in two or more cities also may be inconvenienced by unessential differences in regulations. Uniform regulations that are accepted generally as adequate simplify the sale of milk in distant markets when supplies there are inadequate, although there is a tendency to be less critical when supplies are short.

The ordinance and code discourages the use of public health regulations to establish unwarranted trade barriers against acceptance of high-grade milk from other milk sheds. These may be encouraged by local producers and/or processors to avoid competition. Regulations requiring that milk be produced or processed within a certain distance of the city where it is sold definitely are discriminatory and unwarranted.

Essentials in Milk Supervision. The important points in milk supervision are a sound program, such as that provided by the ordinance and code, and a trained personnel to implement it. Adequate milk supervision requires persons sufficiently familiar with milk problems to detect irregularities in production, processing, and handling methods and to advise producers and processors in the correction of any irregularities that develop. This requires training and experience along various lines, including the interpretation of laboratory and

field inspection data. Without the proper personnel, the funds spent on milk supervision largely may be wasted.

Interpretation of Bacterial Counts. Proper interpretation of bacterial counts on either raw or pasteurized milk gives significant information that is very useful to regulatory officials and plant management in detecting unsatisfactory methods. It should be recognized, however, that there are limitations in both plate and microscopic counts on milk so that results of either method represent estimates rather than actual numbers. Results of counts by a supervising agency should be confidential information between the agency and the farm or plant involved, unless legal action follows, when publication may be necessary. Publication of a series of counts on satisfactory supplies, for interpretation by consumers who do not appreciate the limitations of the procedures employed and who may attach unjustified significance to small differences in counts, sometimes has resulted in a purchaser changing supplies because the new distributor was reported to have an insignificantly lower count than the old.

REFERENCES

1. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 99 (1918).
2. *J. Dairy Sci.*, **2**, 487 (1919).
3. *J. Dairy Sci.*, **3**, 540 (1920).
4. *Ill. Agr. Expt. Sta. Bull.* 204 (1918).
5. *J. Hyg.*, **43**, 96 (1943).
6. *J. Milk and Food Technol.*, **10**, 319 (1947).
7. *Cornell Agr. Expt. Sta. Bull.* 867 (1950).
8. *Standard Methods for the Examination of Dairy Products* (Last ed.). American Public Health Association, Inc., New York, N. Y.
9. *J. Milk Technol.*, **2**, 4 (1939).
10. *J. Milk Technol.*, **1**, 11 (1938).
11. *Am. J. Public Health*, **30**, 273 (1940).
12. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 263 (1942).
13. *Ill. Agr. Expt. Sta. Bull.* 495 (1943).
14. *Public Health Repts.*, **56**, 944 (1941).
15. *Food Eng.*, **25** (1), 82 (1953).
16. *J. Milk and Food Technol.*, **16**, 77 (1953).
17. *J. Dairy Sci.*, **34**, 804 (1951).
18. *Milk Dealer*, **41** (12), 146 (1952).
19. *Texas Agr. Expt. Sta. Bull.* 770 (1953).
20. *Food Eng.*, **25** (5), 75 (1953).
21. *Can. Dairy Ice Cream J.*, **27** (9), 86 (1948).
22. *J. Infectious Diseases*, **7**, 377 (1910).
23. *B. W. Hammer Panegyric*, 155, 1937. Collegiate Press, Inc., Ames, Iowa.
24. *Refrig. Eng.*, **45**, 334 (1943).
25. *J. Dairy Sci.*, **30**, 1371 (1947).
26. *J. Milk and Food Technol.*, **15**, 181 (1952).
27. *J. Milk and Food Technol.*, **16**, 213 (1953).
28. *J. Milk and Food Technol.*, **18**, 32 (1955).
29. *J. Milk and Food Technol.*, **17**, 214 (1954).
30. *Natl. Acad. Sci., Natl. Research Council, Publ.* 250 (1953).

Pathogens in Dairy Products

9

Spread of diseases through milk and its derivatives constitutes the most important problem involving the bacteriology of these products. The prime consideration with any food material is its safety, and, when a product is used that may contain pathogenic organisms, its bacteriological condition is of special concern. Many of the present practices in the preparation and handling of dairy products are the result of attempts to prevent dissemination of pathogens through them.

Historical. The first important evidence of the spread of disease through milk was obtained by Dr. Michael Taylor [1] of Penrith, England, in 1857. This was before the science of bacteriology had been developed and before any organism had been proved the cause of a disease. Taylor observed a number of cases of typhoid fever that had only a milk supply in common. A servant girl had returned to her family in Penrith suffering from typhoid fever. She lived with her parents and 4 other children in a cottage consisting of 2 poorly ventilated and lighted rooms. The father kept 3 cows and sold milk to about 14 families. The mother milked the cows and distributed the milk from the kitchen. Two of the other children in the family developed the disease and the mother nursed all 3 patients while conducting the milk business. As soon as the servant girl was convalescent she helped with the milk distribution. Thirteen cases of typhoid

occurred among 7 families obtaining milk from the cottage, and from these primary cases a number of others developed. Taylor concluded that the milk was infected in the kitchen from the typhoid patients being nursed there.

Apparently, little immediate attention was given to the important possibilities suggested by the observations of Taylor. In 1867, an outbreak of scarlet fever occurred in Penrith and also was studied by Taylor [1] who concluded that the disease was spread through milk as a result of its infection during the handling.

Evidence that man may acquire infectious material from diseased cows through consumption of milk developed along somewhat different lines. In 1865, Villemin proved experimentally the infectious nature of tuberculosis by inoculating tuberculous material from man into rabbits and then soon afterward found that tuberculous material from cows also produced the disease in rabbits. The inoculability of tuberculosis suggested its spread through milk, and this later was proved by experiments in which various investigators produced the disease in animals by feeding milk from tuberculous cows.

The pioneer investigations showing that diseases can be spread through milk have been extended until hundreds of cases of various diseases have been traced to milk supplies. Such instances have been reported from all the important countries of the world, and evidence showing the relationship of milk to the cases has been obtained by many different observers.

Importance of Dairy Products Other than Milk. Spread of diseases through milk suggests that products derived from it also are of importance in this connection, although manufacturing methods may have a significant effect on the organisms involved. Ice cream has caused a number of epidemics and young cheese has caused some extensive ones; butter has caused few recorded outbreaks of disease. With cheeses and butter, the extended holding and wide distribution often make it difficult to trace diseases to them.

Spread of Diseases among Farm Animals. Infected milk and milk derivatives also are of importance in the spread of diseases among farm animals and have caused serious economic losses. Some of the early regulations intended to prevent the spread of pathogens through dairy products, applied to milk returned to farms for animal feeding.

Decrease in Spread of Diseases through Dairy Products. Recognition of the importance of dairy products in the spread of diseases led to various control measures, and at present milk and milk derivatives are much less common sources of pathogens than formerly. Impor-

tant advances have been made in eliminating diseases from producing herds. Pasteurization of milk and cream for direct consumption, and also for manufacturing purposes, is practiced widely. Attempts are made to prevent direct infection of dairy products from human sources by medical examinations of plant employees and by developing methods of handling pasteurized milk in plants so there is little direct contact between the product and the employees. Water supplies have been improved and returned containers are adequately treated to destroy any pathogens present. All these procedures have been significant factors in reducing the spread of diseases through dairy products, and there now is little danger of contracting disease from an adequately controlled supply.

According to Armstrong and Parran [2] milk-borne epidemics in the United States reached a maximum in 1914 when there were 55 reported (238 for 1911–1915, inclusive). Since then there has been an encouraging decrease, but milk-borne epidemics still occur, which indicates the need for extension of adequate supervision.

Groups of Diseases Spread through Milk. Diseases that are spread through milk fall into two groups: Group 1, those in which the infectious material comes primarily from the producing animals, and group 2, those in which the infectious material comes primarily from human sources, either directly or by being carried in water, on utensils, etc. Group 1 includes diseases affecting both man and farm animals and one or more apparently affecting only farm animals; group 2 includes diseases that, for the most part, naturally affect only man. The diseases in group 2 and certain of those in group 1 often appear in epidemic form.

GROUP 1. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES PRIMARILY FROM THE PRODUCING ANIMALS

Tuberculosis

The danger in consuming milk from tuberculous cows was suggested by the results of a number of early investigators. In 1882, Koch [3] reported the discovery of the organism causing tuberculosis and then, after extensive study, concluded that bovine and human tuberculosis are identical. However, the results of Smith, published in 1896 [4] and 1898,[5] showed that there are important differences between the organism commonly causing the disease in man and the one responsible for the disease in cattle and laid the foundation for a differentiation

into the human and bovine types. The work of Smith at once raised the question of the importance of the bovine type of *Mycobacterium tuberculosis* as a cause of tuberculosis in man. At the British Congress on Tuberculosis in 1901, Ravenel presented evidence indicating that the bovine organism affects man and Koch reported that, on the basis of further experimental work, he no longer believed that the bovine organism was transmissible to man. The controversy thus begun led to extensive investigations in many countries, and these show definitely that man may contract tuberculosis from diseased cattle.

Bovine Organism in Human Tuberculosis. Because tuberculosis has been so largely eliminated from dairy herds and also because pasteurization is so widely practiced, the bovine organism now is of relatively little importance as a cause of human tuberculosis in the United States. At one time, however, it was of considerable significance. The work of Park and Krumwiede [6] in New York City, published in 1911, showed that the bovine organism was responsible for 1 or 0.33% of 306 cases of tuberculosis in adults (over 16 yr); for 9 or 16.36% of 55 cases in persons 5 to 16 yr; for 25 or 21.55% of 116 cases in persons under 5 yr; and there was 1 double infection. If all the cases are considered, the bovine organism was responsible for 7.34%.

Park and Krumwiede,[7] in 1912, tabulated data (including their own) obtained by investigators in various countries. Some of the studies involved only a few cases and often these were selected because of the probability of the bovine organism being involved. The tabulation again indicates the importance of the bovine organism in human tuberculosis when control measures are uncommon. It also shows that its incidence varies with the location of the lesion and that it rarely causes pulmonary tuberculosis.

In a group investigation [8] during the years 1943–1945 on nonpulmonary tuberculosis in England (Report to Medical Research Council), 26% of the strains of tubercle bacilli isolated from 994 patients belonged to the bovine type and 74% to the human type. A history of drinking raw milk was given by 87% of patients infected with the bovine organism and 53% of patients infected with the human organism. Analysis of the frequency distribution of bovine infections at different ages showed that, contrary to earlier reports, the maximum proportion of infections occurred in the 5 to 9 yr group instead of the 0 to 5 yr group. This was attributed to gradual replacement of raw milk by dried, boiled, and pasteurized milk for infant feeding and to introduction of milk at schools, which resulted in many children

consuming raw milk. About 30% of infections with the bovine organism occurred in persons over 15 yr. Following a similar survey in Wales, it was estimated that the deaths, through nonpulmonary bovine tuberculosis, of 1,300 to 1,400 persons in England and Wales during 1944 might have been prevented if there had been an adequately pasteurized milk supply.

The danger to consumers when living bovine organisms are present in milk is well established by various additional investigations. For example, Price [9] studied 300 tuberculous children under 14 yr in Toronto and found that 15% of the extra-pulmonary tuberculosis (tuberculosis of bones, joints, lymph nodes, kidneys, and skin) was due to the bovine organism. The children infected with this type came to Toronto from widely scattered areas where there was no pasteurization, and each child had consumed raw milk. The evidence definitely pointed to the alimentary route of infection. In three instances the bovine organism was demonstrated in the milk supply consumed by a child. A small outbreak of bovine infection occurred among children supplied by one dairy distributing raw milk. No cases of bovine infection were detected in children from Toronto where pasteurization had been enforced for an extended period. Children infected with the human organism gave histories of contact with human cases.

***M. tuberculosis* in Milk.** Soon after *M. tuberculosis* was isolated, the examination of milk for it began. In 1927, Tonney *et al.*[10] compiled data on 16,700 samples of milk that had been studied in various countries; 1,448 or 8.66% were positive. In some of the more important cities in the United States during the period from 1907 to 1925, the percentages of the samples of milk that contained *M. tuberculosis* varied from 2.7 to 44.0; most of them were in the range from 2.7 to 15.9. On the basis of the findings, it was estimated that during the 3 yr prior to Jan. 1, 1926, approximately 43,750 qt of infected milk were sent each day to the plants pasteurizing milk for the Chicago market. The organisms obtained from the positive samples were all of the bovine type.

More recent data show that in certain countries a considerable percentage of milk samples still contain tubercle bacilli.[11,12]

Examination of Milk for *M. tuberculosis*. Three methods are used in the examination of milk (or preferably sediment and cream centrifuged from it) for *M. tuberculosis:* (1) Microscopic examination, (2) culturing under special conditions, and (3) animal inoculation.[13]

Ordinarily, animal inoculation is the most satisfactory. The methods are readily adaptable to the examination of other dairy products.

In examining a milk supply for *M. tuberculosis*, one negative result means very little because of the intermittent infection of milk, and several examinations extending over a considerable period are advisable. Moreover, absence of the organism in a milk supply at one time means nothing as far as the future is concerned, and when tuberculosis is present in the producing animals the milk suddenly may contain the organism. If raw milk free from *M. tuberculosis* is desired, it can be assured only by keeping the cows free of the disease.

Methods by Which Milk from Tuberculous Cows Is Infected. Infection of milk from tuberculous cows with *M. tuberculosis* is influenced greatly by location of the lesions and their general character.

When there is a lesion in the udder, the milk commonly contains the causative organism. Briscoe and MacNeal [14] tabulated data reported in the literature and found that, of 39 samples from visibly diseased udders, 34 (87.2%) were positive; most of the investigators reported results on only a few samples, owing no doubt to infrequency of the condition. Moore [15] noted in an examination of 87 samples of milk from individual animals that the 2 positive samples came from cows having udder infections. Milk from tuberculous udders often contains such large numbers of *M. tuberculosis* that many organisms can be seen in each field of a microscopic preparation made with sediment or cream from the milk.

Some of the tuberculous cows without infected udders give milk containing *M. tuberculosis*, others do not. Results compiled from the literature [14] on the presence of the organism in the milk of tuberculous cows having no recognizable udder lesions showed that, of 748 cows studied, the milk of 131 (17.5%) was positive. Some of the samples were drawn carefully; with others there was the possibility of infection after the milk left the udder. *M. tuberculosis* can get into the milk of tuberculous animals without udder lesion mainly when (1) the organism is present in the blood,[16] (2) particles of infected manure fall into the milk, and (3) material from an infected uterus enters the milk.

***M. tuberculosis* in Dairy Products Other than Milk.** With *M. tuberculosis* in milk, it also would be expected in other dairy products, and it has been found in them in various parts of the world. At present, in the United States butter, cheeses, and ice cream are of no importance as a source of *M. tuberculosis*.

Control of Tuberculosis from Dairy Products. Cases of human tuberculosis due to the bovine organism in dairy products are controlled readily through elimination of tuberculosis from dairy herds and adequate pasteurization of milk and cream. Although the products may be infected after pasteurization with the human organism from tuberculous employees, this appears to be a minor consideration with the present control over the disease in persons.

Introduction of Tuberculosis into a Herd. There are two common methods by which tuberculosis is introduced into a herd: (1) Through purchase of tuberculous animals, and (2) through return, for feeding purposes, of infected dairy plant by-products.

The danger of introducing a tuberculous animal into a herd through purchase is so great that a careful herdsman will not bring an animal into his herd until its freedom from the disease definitely has been established. Legislation recognizes the possibilities along this line and prevents some of the undesirable practices.

Return of infected dairy plant by-products has been an important factor in the spread of tuberculosis among farm animals. Russell,[17] in 1907, reported observations on the infection of cattle in this way. He noted that the percentage of animals reacting to tuberculin in the neighborhood of 2 Wisconsin creameries was higher than the average and also that the young stock raised on the different farms was infected, whereas often the mature animals, many of which were brought into the neighborhood, were not. The general situation is illustrated by data from one of the creameries. Of the 43 patrons, 36 had their herds tested and 33 contained reactors, with 12 herds showing more than half the animals diseased. A total of 784 animals was tested and 271 reacted. Of the 3 herds not infected, the owners of 2 had moved into the locality within the year and brought all their animals with them. One farmer, who also had come into the section within the year, had 2 reactors in a herd of 28, but these had been bought from other herds. Another farmer, who had only 1 reactor among 48 animals, was a cream patron, and the reactor was the only animal in the herd that had been fed on skim milk from the creamery.

Information on the presence of *M. tuberculosis* in the milk of infected cows and in the various derivatives of such milk has resulted in extensive legislation requiring pasteurization of dairy plant by-products returned to the farms for animal feeding.

Financial Losses Due to Tuberculosis in Cattle. Although the primary interest in tuberculosis in cattle is due to its public health relationships, economic considerations also are involved. With tuber-

culous herds there may be financial losses due to occasional death of an animal, decreased milk production, exclusion of animals and milk from certain markets, and infection of hogs associated with them.

Brucellosis

Brucellosis in man is acquired primarily from animals infected with one of the organisms belonging to the genus *Brucella.* The species involved are *Brucella melitensis* (caprine type), *Brucella abortus* (bovine type), and *Brucella suis* (porcine type); the three species are closely related, but *Br. melitensis* and *Br. suis* are more highly infectious for man and cause a more severe form of illness than *Br. abortus.* Each of the three species may produce disease among various farm animals. Man is infected directly through contact with diseased animals or their tissues or discharges, or indirectly through consumption of raw milk and milk derivatives from certain diseased animals. The disease in man often is referred to as undulant fever.

Feig [18] noted that the *Brucella* strain most frequently involved in the production of brucellosis in man in a given area is determined by the nature of the specific live stock industry there. Thus, in Mexico (with many goats) 95% of the isolations from man were *Br. melitensis;* in Minnesota and Wisconsin (with many cattle) 86 and 96%, respectively, were *Br. abortus;* and in Iowa and Alabama (with many hogs) 63 and 76%, respectively, were *Br. suis.* Various factors other than the simple numerical distribution of goats, cattle, and hogs may influence this relationship.

Brucellosis from Goats. Brucellosis has existed for an indefinite period on the island of Malta. The causative organism was discovered by Bruce,[19] in 1887. Because of the prevalence of the disease among soldiers and sailors stationed on the island, the British government appointed a commission to investigate the sources of infection and advise methods of control. As early as 1905, the commission considered that milk from goats was an important factor in the spread of the disease among persons. Subsequent studies indicated that goats were infected easily, about 50% of them acquired the disease naturally, and the causative organism was eliminated in the milk and urine. Ten per cent of the goats in Malta were found to be eliminating the organism in milk, and this was highly infectious for monkeys, in which it produced attacks similar to those in man.

In the United States it first was established through agglutination tests and blood cultures that brucellosis existed endemically in the goat-raising sections of Texas. The human cases reported there gave

a history of association with the goat industry, and some had consumed goats' milk.

Importance of goats' milk in spreading the disease has been shown in various ways. When the fresh milk supplied the soldiers at Malta was pasteurized, the disease among them was reduced 90%. The disease did not occur among the convicts of the civil prison at Malta who were not allowed milk. It was practically eliminated among British soldiers stationed at Gibraltar by prohibiting the consumption of raw goats' milk. Mexican goat herders are only infrequently infected; this may be because they boil milk before drinking it.

History of a Shipment of Goats. The history of goats shipped from Malta to the United States [20] indicates the dangers of raw milk from infected animals. Consumption of the milk caused brucellosis among persons on board ship and in at least one person after the goats reached the United States. Examinations resulted in recovery of the *Brucella* organism, first from the milk of two goats and later from that of several more.

Epidemic of Brucellosis Due to Goats' Milk. Lake [21] reported an epidemic of brucellosis that occurred in Phoenix, Ariz., as a result of consumption of goats' milk. More than 30 cases were positively diagnosed and undoubtedly many others occurred. In all but 3 of the cases goats' milk from the same diary was known to have been used and in the 3 it could not be excluded. A *Brucella* organism was recovered from 2 of the cases. Agglutination tests were made on 115 goats in the herd responsible for the epidemic and 18.3% were positive.

Brucellosis from Cattle. Contagious abortion in cattle has been known in England and Continental Europe for many years. In 1897, Bang [22] ascribed it to an organism which produced the disease on artificial inoculation and could be recovered from the infected animals. The organism now is designated *Br. abortus* and is the common cause of abortion in cattle. In 1918, Evans [23] noted the close relationship of *Br. abortus* to the organism described by Bruce, and this suggested the possible relationship of *Br. abortus* to disease in man, a relationship which has been confirmed repeatedly.

***Brucella* Organisms in Cows' Milk.** Various investigators have observed that guinea pigs inoculated with cows' milk for the purpose of detecting *M. tuberculosis* and autopsied after extended periods showed lesions which could be mistaken easily for tuberculosis but in which no acid-fast organisms could be demonstrated. The responsible organism eventually was cultivated [24] and identified [25] as the organism isolated by Bang.

Many studies show that milk from herds infected with *Br. abortus* frequently contains the organism. It can become established in the udder, multiply there, and then appear in the milk, often in relatively large numbers. The udder presumably can be infected from the genital tract by discharges that contain the organism; transmission through the blood stream is another possibility. The percentage of samples of milk from infected cows that contain *Br. abortus* often is high; Gwatkin [26] examined 102 such samples and found 52 positive. *Br. abortus* also can get into milk from the stable surroundings. Results of Cameron [27] indicate it can survive for extended periods under a variety of conditions.

Dangers from Infected Cows' Milk. Ingestion transmission probably causes fewer cases of brucellosis in man than contact with diseased animals and tissues, but infected raw milk apparently causes a significant number. However, the number is small when the distribution of the disease among cattle and the frequency with which infected cows eliminate the causative organism in the milk are considered. Hardy *et al.*[28] suggested that dairy products which serve to disseminate the infection carry either large numbers of organisms or strains of unusual virulence and noted that relative human immunity also must be operative. Many human cases of brucellosis are isolated or in small groups, and it appears that only a small percentage of persons consuming an infected milk supply develops the disease.

Various studies indicate that infected cows' milk may be responsible for human brucellosis. Stoenner *et al.*[29] reported that, of 25,294 human sera examined in Utah in 1947 and 1948, 2.28% reacted positively to *Br. abortus*. High agglutination titres were more common in rural districts, where raw milk rather than pasteurized milk was consumed and where there was more contact with animals, than in urban districts. Also, high titres were more common in individuals with a history of raw milk consumption than in those with a history of pasteurized milk consumption. In 24 of 54 cases investigated, human cases could be traced to cows currently infected.

With 268 brucellosis patients in Minnesota between 1945 and 1948, Magoffin *et al.*[30] found *Br. abortus* was the causal organism in 85.8%, *Br. melitensis* in 8.2%, and *Br. suis* in 6%. Of 255 patients, 121 (47.45%) gave a history of contact with cattle and hogs plus use of raw milk, while 63 (24.72%) had ingested raw milk or cream without known exposure to other sources of infection. In several of the latter cases evidence of brucellosis among cows supplying the milk was obtained.

Feig [18] noted that, of 190 noncontact cases in Wisconsin, only 107

(56%) reported that they used raw dairy products, the others claiming they used only pasteurized dairy products. It was suggested that there is need for further epidemiological study of methods of transmission with both contact and noncontact cases.

Brucellosis from Hogs. *Br. suis* apparently was first isolated in 1914 from premature fetuses from sows. The usual cause of human infections with *Br. suis* is contact with infected hogs or tissues from them on farms and in meat-packing plants. However, in some cases *Br. suis* becomes established in the udders of cows and may cause disease in humans as a result of consumption of milk from the udders. Washko and Hutchings [31] produced *Br. suis* infections in 10 lactating cows by introducing a suspension of organisms into the udder, via the teat canal. In some cases acute mastitis developed and *Br. suis*, which was eliminated in the milk, was considered the causal agent.

Epidemics of Brucellosis Due to *Br. suis*. Beattie and Rice [32] studied an outbreak of milk-borne brucellosis that was caused by *Br. suis*. It involved 30 persons of whom 27 obtained milk from one dairy. The dairy had 20 cows and supplied about 80 households; in 18 of these, brucellosis occurred. The last case appeared 13 days after sale of milk from the dairy was stopped. *Br. suis* was obtained in blood cultures from 6 of 14 patients and from the milk of 1 of the cows in the herd.

A milk-borne outbreak of brucellosis due to *Br. suis* occurred in a home for elderly persons in Connecticut.[33] It involved 14 cases with 3 deaths out of a population of 305 inmates and 81 employees. The evidence indicated the disease was due to drinking raw milk from cows infected with *Br. suis*. The organism was isolated from the blood of 2 patients and an abscess in a third. There was opportunity for the infection of the cattle from a herd of infected swine.

Examination of Milk for *Brucella* Organisms. Milk, or cream from it, can be examined for *Brucella* organisms [13] by (1) animal inoculation, (2) culturing, using crystal violet in the medium, (3) testing for the presence of agglutinins, and (4) the ring test. The ring test has some important advantages for certain purposes and also some limitations. The *Brucella* species can be differentiated in various ways; [13] the dye method, which utilizes differences in growth behavior of the species on solid media containing certain dyes, has been accepted widely.

Control of Brucellosis from Dairy Products. Although adequate pasteurization controls the spread of brucellosis through dairy products, elimination of the disease from dairy herds also is desirable and

progress along this line has been rapid. The economic importance of brucellosis in cattle is another factor in the elimination program.

Q Fever

Coxiella burnetii, the etiological agent in Q fever, is distributed widely in nature where it causes inapparent infections in various species of animals. Since the early observations on Q fever in man were reported from Australia and Montana, the disease has been recognized in various countries. The first naturally occurring outbreaks in the United States involved considerable numbers of cases in packing-house employees in Texas and Illinois.[34] Shortly thereafter the disease was found to be endemic throughout California and also present in other states.

Cows, goats, and sheep eliminate *C. burnetii* in their milk, and this represents one method of transmitting the disease to man. Enright *et al.*[34] suggested that this method of transmission needs clarification and that studies have not indicated infected milk as being of primary importance in the spread of the disease to man; other suggested routes of transmission are the air-borne route and contact with contaminated meat, hides, hair, and wool.

Role of Livestock in Human Q Fever. Beck *et al.*[35] examined the possible sources of infection in 300 human cases of Q fever which occurred in Los Angeles and the surrounding area. Information was obtained on 286 cases, and 78% gave a history of one or more of the following: (1) Living close to dairies or livestock yards; (2) having direct contact with cattle, goats, and sheep or their raw products; or (3) being regular consumers of raw milk. The last represented 32% of all the cases. Huebner and Bell[36] noted that epidemiological studies of nearly 10,000 cases gave evidence that raw milk was responsible for a considerable proportion of human infections with *C. burnetii* in the Los Angeles area. A review of studies on Q fever in northern California by Lennette and Clark[37] indicated that raw milk is not a common vehicle for transmission of Q fever there. From the results of tests on blood sera of over 16,000 dairy cattle, goats, and sheep, Lennette *et al.*[38] concluded that in the endemic area of southern California, which supports a dense dairy population, these animals may play an important role in the spread of Q fever; in northern California goats and sheep appeared to play the corresponding role.

***C. burnetii* in Cows' Milk.** Irons *et al.*[39] found *C. burnetii* in two raw milk samples from widely separate dairies in Texas. Jellison *et al.*[40] autopsied a cow which had been shedding *C. burnetii* for a

long period. The milk and udder tissues of all four quarters were found infected with the organism; the lymph nodes near the mammary gland also were positive, but all other tissues and fluids were negative. Inoculations of *C. burnetii* into the udders of lactating cows by Bell *et al.*[41] resulted in the development of Q fever in the animals. The organism was isolated from the milk of the animals up to 200 days after inoculation. Cows inoculated with large doses of *C. burnetii* developed acute mastitis which generally subsided by about the eighth day after inoculation, but the animals continued to shed the organism in their milk for long periods.

In the experiments of Stoenner,[42] in which the teat orfices of lactating cows were exposed repeatedly to Q fever infected milk during milking, results indicated that hand milking is not an important factor in the spread of Q fever among cows with normal mammary glands.

Infection from Goats. Clark *et al.*[43] described an outbreak of Q fever involving 15 out of a crew of 52 aboard a ship transporting dairy goats. Evidence of infection with *C. burnetii* was obtained from blood sera of a high percentage of the goats. Milk samples were not examined, but goats' milk was used by a high proportion of the crew. Lennette and Welsh[44] isolated *C. burnetii* from the milk of 3 of 31 lactating goats, as well as from the air of the milking shed.

Infection from Sheep. Jellison *et al.*[45] traced a human case of Q fever to sheep. The milk from one flock was found to be carrying *C. burnetii.*

Effect of Q Fever on Composition of Milk. The changes that occurred in the composition of the milk following artificial infection of the bovine udder with *C. burnetii* were found by Armsbee[46] to differ from those associated with chronic mastitis. Fat, solids-not-fat, total nitrogen, noncasein nitrogen, chlorides, and *p*H were increased, whereas casein, lactose, and water were decreased; milk yield also was decreased.

Foot-and-mouth Disease

Foot-and-mouth disease is a highly infectious disease that affects various domestic and wild animals, especially cattle, and sometimes is transmitted to man. The virus is present in the fluid of the vesicles and, from this source, may gain entrance to saliva, feces, urine, and milk. Lesions sometimes occur on the udder; in certain instances the milk ducts and even the secreting portions of the udder are involved, and the milk then is highly infectious.

Foot-and-mouth disease causes a reduction in the milk flow in cows,

and the milk produced may be very abnormal in appearance and flavor. Wing [47] reported that the daily milk production of an infected herd dropped from 4,000 to 1,200 lb in 6 days.

The disease in man may be contracted by contact with diseased animals or ingestion of infected dairy products. In European countries, where foot-and-mouth disease has been common, many cases in man have been reported, sometimes in epidemic form. Children appear to be especially susceptible. The disease in man usually is mild although occasionally the symptoms are severe and some cases end fatally.

Various outbreaks of foot-and-mouth disease have occurred in the United States, primarily among cattle, but these have been stamped out by quarantine and destruction of infected herds. Occasional cases of foot-and-mouth disease in man have occurred in the United States.[48,49] In an outbreak in California, no cases of foot-and-mouth disease occurred in man under conditions which appeared favorable for its spread, since before the disease was recognized raw milk from infected farms was sold.[47]

Milk or milk derivatives are a means of spreading foot-and-mouth disease from one farm to another. Wing [47] recorded an instance in which milk from an infected herd was separated and the raw skim milk shipped to another farm, where it caused foot-and-mouth disease in hogs to which it was fed; the disease then spread from the hogs to cattle. There was no connection other than the milk between the two farms, which were 57 miles apart.

Johne's Disease

Spread of Johne's disease from one farm to another through dairy products is suggested by the general nature of the condition. Characteristically, portions of the intestinal wall are thickened and contain large numbers of the causal organism so that fecal material is infected readily. Hastings *et al.*[50] pointed out that desquamation of the epithelium probably is rapid in areas in which the organism is growing, and this suggests that shedding of the organism is a steady process. Contamination of milk with infected fecal material would add the organism to the milk, and its transfer to other farms could spread the disease in the way that tuberculosis is spread. Feeding infected milk to calves on the farm where it is produced also may be a factor in extending the disease.

Lovell *et al.*[51] reported that, when exposed to atmospheric condi-

tions, feces naturally infected with the organism causing Johne's disease may contain cells that are viable for as long as 246 days.

Anthrax

Bacillus anthracis may be present in the milk of cows that have anthrax. M'Fadyean [52] examined 3 samples of milk drawn postmortem from cows that had died of anthrax and, in each, anthrax bacilli were so numerous they were detected readily by microscopic examination. In an outbreak studied by Moore [53] the organisms were not found in the milk until just before, or soon after, death of the cows. About 14 animals died and for 3 weeks after the first case appeared, and before the diagnosis was made, milk from the herd was retailed without injury to consumers. It was suggested that elimination of organisms with body discharges may afford an additional opportunity for infection of milk. Morris [54] reported that *B. anthracis* remained virulent for 10 yr in milk taken from the udder of a cow that died of the disease.

It is probable that the milk of cows having anthrax is not infected with *B. anthracis* until active multiplication of the organism in the blood stream begins. This usually occurs only shortly before death and is accompanied by a temperature increase and other evidences of an abnormal condition, among which there ordinarily is suppression of milk secretion and change in its character. Accordingly, there is little danger from the milk of a cow having anthrax until the disease has progressed to a point where it is very evident.

Actinomycosis

The possibility of man acquiring actinomycosis through consumption of milk from infected cows is very slight. If the udder is involved the danger from *Actinomyces bovis,* as well as from any secondary invaders present in the lesions, presumably is greater than when the lesions are in other parts of the body. Cases of actinomycosis in man sometimes appear to be the result of infection through injuries to the mucous membranes of the intestinal tract, but the organism probably must be carried into the tissues.

Actinomycosis of the udder is uncommon in the United States. Kendall [55] reported a number of cases among the cows in Victoria that may have been the result of an unusual prevalence of the causative organism on the pastures; the hind quarters were involved more frequently than the fore quarters. Albiston [56] concluded from a review of the literature and his own work that true actinomycosis of the

udder is very rare. Examination of a small number of milk samples from cows showing clinical signs of mammary actinomycosis did not reveal any indication of infectious material from the nodules.

Rabies

Although cows occasionally develop rabies there is little danger of spread of the disease through milk. Peters [57] reported that on several occasions inoculation of rabbits and guinea pigs with milk from a rabid cow had given negative results. Nicolas [58] found that milk and mammary tissue of herbivorous animals suffering from rabies never was virulent. Mugrage [59] noted that a small series of rabbits, either injected with milk from rabid does or nursing these does, did not develop rabies. He suggested that pasteurization, as ordinarily conducted, may not destroy rabies virus. Hardenbergh [60] stated that one authority had observed a doe that developed rabies while nursing her young; the little rabbits were kept for more than 1 yr and remained healthy.

Trembles and Milk Sickness

Trembles is a disease of cattle and other farm animals. In certain cases the consumption of milk or milk derivatives from diseased animals causes a condition in man that is referred to as milk sickness. The disease in animals and man has been known for many years and was encountered by early settlers in many sections of the United States. Certain areas were regarded as milk-sick areas and were avoided, in some instances by fencing. As the country was settled the disease gradually decreased until now it has disappeared in areas where once it was common. As late as 1918, it was noted [61] that the disease probably occurs in the mountainous portions of all the counties of western North Carolina and in the immediately adjacent sections of states having similar conditions. In 1926, Marsh *et al.*[62] reported that a disease of cattle, commonly called alkali poisoning or milk sickness, had long been known in the Pecos Valley of New Mexico and Texas and that a disease in man was identified with it.

The theories as to the cause of trembles and milk sickness have been along three lines, mineral poisons, micro-organisms, and poisonous plants. Certain plants now generally are regarded as responsible.

Plants Responsible for the Disease. Poisonous plants were considered to be the cause of the disease by some of the early observers. This theory was supported by various facts, such as period of the year the disease is most common and its disappearance as the land is culti-

vated. Although a number of plants have been accused, white snakeroot or richweed (*Eupatorium urticaefolium*) has been blamed most often. In 1917, results of various investigations were reported which definitely established that one plant which causes the disease is white snakeroot. Curtis and Wolf [63] developed 15 cases in sheep by feeding the plant, with 14 resulting fatally; death occurred in 5 to 27 days following beginning of the feeding. Marsh and Clawson [64] found that white snakeroot was toxic for both cattle and sheep. Moseley [65] noted that an ether extract of the leaves of white snakeroot, when consumed by rabbits, affected them similarly to the leaves themselves. In 1918, Wolf *et al.*[61] reported an extended series of experiments in which they produced disease in various animals by feeding white snakeroot. The investigations were carried out where no case of the disease ever had appeared prior to the trials, and the white snakeroot was shipped 400 miles. Two lambs contracted the disease by suckling their mothers, proving that the disease can be transmitted through milk. Animals appeared to differ greatly in their susceptibility to poisoning with white snakeroot. Sackett [66] reported feeding experiments with white snakeroot and various extracts of it which indicated that guinea pigs are more resistant to poisoning than rabbits. The viscera and flesh of rabbits that had died from the poisoning were not harmful to a cat.

Studies on the milk sickness encountered in the Pecos Valley [62] showed that typical symptoms could be produced in cattle, horses, and sheep by feeding sufficient quantities of rayless goldenrod, or jimmy weed (*Aplopappus heterophyllus*). The toxic substance may be excreted in the milk and calves and lambs poisoned in this way. The findings suggest that cases in man may occur from consumption of milk or butter from cows feeding on rayless goldenrod.

Toxic Constituent of White Snakeroot and Rayless Goldenrod. Investigations of Couch [67] indicate that tremetol is the constituent of white snakeroot that is responsible for milk sickness. The compound has the properties of an unsaturated alcohol and is soluble in fat and fat solvents; it may be secreted in milk and, because of its solubility, would be carried into butter. When white snakeroot is dried, the tremetol content rapidly decreases and the completely dried plant is not toxic. The tremetol content of white snakeroot from different sections varies widely, and in some areas the plant apparently is not dangerous. Later,[68] it was reported that tremetol also is the toxic constituent of rayless goldenrod and that the dry plant appears to lose

its toxicity slowly, the process being much less rapid than with white snakeroot.

Distribution of Cases of Milk Sickness. Couch [69] pointed out that milk sickness is primarily a rural disease. Outbreaks have occurred principally on farms and in small towns where persons often consume milk from one or a few cows. In cities, the milk comes from many sources, and diluting milk from cows having trembles with normal milk ordinarily renders it harmless. Tremetol is destroyed only slowly by the temperature of boiling water [69] so that pasteurization probably affects it only slightly.

Mastitis

The relationship of organisms to mastitis was recognized early as a result of the development of an infection when materials containing organisms were injected into the udder through the teat canal, and by the frequent appearance of an inflammation when a normal udder was injected with material from one that was diseased.

General Condition. Variations occur in the extent and type of udder infections because of differences in resistance of various animals and in virulence of the causative organisms. In severe mastitis the affected quarter may be intensely inflamed, feverish, and indurated, whereas in mild cases the abnormality may be very slight and difficult to detect. Often only 1 quarter is abnormal, but in certain cases 2 quarters or even more are involved. With a severe infection the milk may be abnormal in appearance; with a slight infection it may not. Many infected udders quickly return to a condition that appears normal although the causative organism may persist for extended periods. Occasionally, one or more quarters are so severely involved that milk secretion ceases and that portion of the udder is lost for the time being, or even for succeeding lactation periods also (see p. 49).

Organisms Causing Mastitis. Mastitis is caused by various organisms, and the many studies on the responsible species in different herds have given variable results. *S. agalactiae* appears to be the causal species most often. It is a typical bovine type that is not pathogenic for man. Attempts to infect persons with massive doses of this organism have been unsuccessful; milk containing it frequently is fed to farm animals without causing disease. *S. equisimilis* and *S. uberis* also appear to be typical mastitis species. Micrococci, especially *M. pyogenes* var. *aureus,* have been responsible for many cases of mastitis. Various other organisms have been identified, in-

cluding coliform types, *Ps. aeruginosa*, *Corynebacterium pyogenes*, yeasts, etc. Mixed infections have been noted.

Extent of Mastitis. In many herds mastitis appears to be very common. Spencer [70] made a field survey of 12,077 cows in 731 Illinois dairy herds and found 28.8% of the animals infected with streptococci. In 24 Pennsylvania dairy herds, Reid [71] found the number of mastitis cases per 100 animals per yr ranged from 13.6 to 34.9.

Importance in Human Disease. Occasionally, mastitis is caused by typical human streptococci that gain entrance to the udder directly or indirectly from some person. The organisms sometimes find favorable conditions in the udder and multiply extensively. As a result the freshly drawn milk contains enormous numbers of bacteria that are pathogenic for man and extensive epidemics have been caused in this way. The disease in man most often caused by the human types of organisms coming from the udder is septic sore throat, but there are other possibilities, especially scarlet fever.

In some cases organisms growing in the udder produce toxic substances that cause poisoning when the milk is consumed. Micrococci appear to be responsible most often.

Spread of Mastitis. Organisms causing mastitis frequently are spread from one animal to another in a herd by the hands of the milkers, milking machines, and in various other ways. Mastitis thus presents a problem in herd management. Because of the economic losses involved, as well as public health considerations, attempts frequently are made to free herds of mastitis and maintain them in this condition.

Lancaster and Stuart [72] transmitted *S. agalactiae* infection by hand milking, with hands dipped in a secretion from an infected cow, or by machine milking, with contaminated teat cups. Some animals were very susceptible to repeated experimental infections, whereas others were very resistant. In most cows susceptibility increased with age and/or previous infection. Cunningham and Iverson [73] noted no infections with *S. agalactiae* in 574 heifers in 3 herds within 3 months after calving when mastitis milk was fed to calves.

S. agalactiae survives well in stable surroundings. Kästli and Grossenbacher [74] placed various materials in petri dishes, sterilized them, and then inoculated the organism. It survived 21 to 63 days in feces, 9 to 12 weeks on straw, 15 weeks on cloth, and 15 to 20 weeks in milk.

Destruction of *S. agalactiae* with Chemicals. Studies by Spurgeon *et al.*[75] indicate that complete removal and/or destruction of *S.*

agalactiae on teat cups by dipping in a bactericidal solution might be difficult to attain under practical conditions. However, both hypochlorite and quaternary ammonium compounds removed and destroyed sufficient bacteria to suggest their use for the disinfection of teat cups between cows. More complete destruction of S. *agalactiae* on teat cups was attained by using two successive bactericidal rinses, longer exposures, higher concentrations of bactericide, and inflations free of cracks and checks. Survival of S. *agalactiae* on old worn inflations was much greater than on new inflations.

Of 15 disinfectants studied, Chadkowski [76] found that a quaternary ammonium compound and a synthetic cationic detergent, both of which contained 75% cetyl-trimethyl-ammonium bromide in concentration of 1 to 500 to 1 to 1,000, and an iodine solution in concentration of 1 to 500 to 1 to 800 were most effective against S. *agalactiae*. The quaternary was lethal at 1 to 1,000 in whole milk in 1 min.

GROUP 2. DISEASES IN WHICH THE INFECTIOUS MATERIAL COMES DIRECTLY OR INDIRECTLY FROM MAN

In the United States the most frequent milk-borne diseases belonging to the group in which the infectious material comes from man, either directly or indirectly, have been typhoid fever and septic sore throat. When milk-borne, the diseases in this group often occur in epidemic form in the same manner as when they are spread in other ways. However, milk-borne epidemics have certain characteristics which are helpful in deciding whether the epidemic under investigation is due to milk.

Major Characteristics of Milk-borne Epidemics. The more definite characteristics of milk-borne epidemics and those of most help in detecting infected milk supplies are the following.

1. Cases Are Confined Largely to Users of Milk from One Source. If an epidemic is due to milk from a certain source, the cases are confined largely to persons consuming it. Accordingly, when a considerable percentage of the cases in an epidemic are along the route of one dealer, a milk-borne outbreak is indicated. However, if a dealer furnishes a large part of the milk sold in a community, consumers of the supply may have a high percentage of the cases in an epidemic in which milk is not involved. In general, with the usual complexity in milk distribution, a considerable number of cases among consumers of a certain milk supply, and not among consumers of other supplies, is suggestive.

Various factors tend to limit infections among persons consuming milk from an incriminated supply, such as (1) all portions of the supply causing an epidemic may not contain the responsible organism, (2) in certain homes milk may be heated before use, and (3) persons vary in their resistance to different diseases. The percentage of the persons using an infected supply that contract the disease varies greatly, owing to the above factors. In some instances it is very low and in others it may be nearly 100%. Undoubtedly, infections have occurred through drinking only one glass of milk.

2. Epidemics often Are Explosive in Nature. With milk-borne epidemics of any considerable magnitude, a number of persons acquire the pathogens at about the same time and, with a fairly definite incubation period, a number of cases appear simultaneously. In some instances a given supply is infected over a considerable period and cases then continue to appear; in others the milk is infected only once or twice and all the cases are reported in a comparatively short period.

In addition to epidemics due to milk, those spread through general water or food supplies also may be explosive in nature. Accordingly, the simultaneous appearance of a number of cases of a disease that may be milk-borne suggests a general source of infection, with milk one of the possibilities.

3. Most Cases Are in Homes Consuming Considerable Milk. If an epidemic is milk-borne it would be expected that, with a fairly uniform distribution of the pathogens throughout the supply, homes consuming large quantities of milk would show more cases than those using little milk, provided the milk was not treated by a method which destroyed the causative organism.

4. Most Cases Are among Heavy Milk Drinkers. Heavy consumers of milk have a greater chance of being infected during a milk-borne epidemic than light consumers and, proportionately, more cases occur among them. Children consume much milk and would be expected to show a high case incidence, but the milk they drink often is heated in the homes so that they may show a low incidence.

Minor Characteristics of Milk-borne Epidemics. In addition to the characteristics listed, a number of others that are more or less unusual have been noted in certain milk-borne epidemics. The incubation period sometimes has been rather short for epidemics in which the date of infection of the milk was established. The causes suggested are heavy infections, due to growth of the organisms in the milk, and

rapid absorption of the organisms. Certain epidemics have shown a low mortality but with others the mortality has been normal, or even high. Milk-borne epidemics have been more common in small communities than in cities because often in the former less of the milk is pasteurized. Some of the milk-borne epidemics that have occurred in cities have been very extensive, owing to the wide distribution of the milk.

In milk-borne outbreaks of various diseases there may be a different age distribution of cases than in outbreaks spread in other ways. A relatively high incidence in children has been noted in certain milk-borne outbreaks of typhoid. Godfrey [77] considered that the milk supply should be investigated whenever an unusual number of adult cases of scarlet fever or diphtheria occur in a community.

Methods of Tracing Milk-borne Epidemics. Methods of tracing milk-borne epidemics are epidemiological rather than bacteriological. Isolation of the pathogens from a suspected supply normally is not attempted for the following reasons: (1) Satisfactory methods are not available and failure to find the organism involved in an epidemic is no assurance that it was not in the milk; (2) considerable time is required to examine milk for certain pathogens and in an epidemic a decision as to the source of the causative organism must be made quickly so that control measures can be instituted; (3) the organism responsible for a milk-borne epidemic is not continuously present in the supply and, since the cases must have been infected some time before they began to appear because of the incubation period, a supply may have caused an epidemic and still not contain the responsible organism at the time an examination is attempted.

The usual procedure is to obtain data on each case, directly from the individual or from someone else who is thoroughly informed, and then analyze them in an attempt to locate some source of the organism that is common to all, or the great majority, of the cases. If practically all the cases in an epidemic have used a certain milk supply and have had nothing else in common, the milk is at once under suspicion. The data obtained may point to some source such as water, foods of various sorts, etc., in quite the same way that milk is indicated. A spot map illustrating the distribution of the cases along the different milk routes is valuable when milk is a possible source of the infection since this shows quickly any grouping of cases along a particular route. The point to be considered especially is whether or not a certain milk route shows an excess of cases over the number

to be expected on the basis of the percentage of the total milk furnished.

Care is necessary in obtaining data on the cases since some incident that is not readily recalled may be important in tracing the epidemic. A single glass of milk from a supply other than the usual one may be the only connection between a given case and the milk causing the epidemic.

Tracing an epidemic is essentially an elimination process in which one possibility after another is excluded until the evidence finally points to one remaining source. The possibilities to be considered in an investigation depend on the particular disease involved; for example, the common sources of diphtheria epidemics are quite different from those of typhoid epidemics. Water supplies so carefully controlled that they are known to be safe ordinarily make the detection of milk-borne typhoid easier than when uncertain water supplies must be considered. In reporting to health departments cases of diseases that can be spread through milk, there is an advantage in including the sources of the milk supplies since a tabulation of such data may show at once a case distribution indicating a milk-borne epidemic.

If the findings incriminate a milk supply, valuable confirmatory evidence is obtained through location of the source of the organism, for example, a carrier, a definite case, an infected water supply, etc. When a suspected supply is stopped, or pasteurized if it is raw, and the outbreak subsides, the exact time depending on the incubation period of the disease in question, further proof is provided.

Typhoid Fever

Numerous epidemics of typhoid fever in various countries have been traced to milk and its derivatives, and undoubtedly many others due to these products have been included with those epidemics which have gone unsolved. Milk has been involved most frequently, with ice cream next in importance. Recently, there have been a number of outbreaks of cheese-borne typhoid. In general, they have been due to young cheddar cheese; as a result of them, there now are regulations in certain areas requiring pasteurization of milk for cheese unless the cheese is held for a definite period, the assumption being that this period is long enough for any typhoid organisms originally in the cheese to have died.

Example of Milk-borne Typhoid. An extensive milk-borne typhoid epidemic occurred in Montreal.[78] From March 1 to July 16

there were 5,014 cases, with 488 deaths. Two peaks occurred in the epidemic, one about the middle of March and one about the middle of May. Water supplies were excluded because the distribution of cases was unrelated to the 2 separate and distinct supplies and also because Verdun, an adjoining city with a separate water supply, had many cases. Milk was accepted as the cause of the outbreak because of the age distribution of the cases, 903 being under 10 yr and 718 between 10 and 20 yr, and because a large percentage of the cases used the products of dairy M.D.C. In institutions, such as boarding houses, orphanages, and homes for adults, there was a high case incidence when milk from dairy M.D.C. was used. The supply distributed by dairy M.D.C. came from 1,200 to 1,500 farms and was exposed more or less to a population of 20,000. Some of it came through 4 receiving stations, one of which used water from a polluted river. At the city plant the milk was pasteurized in a machine of an efficient type, but as it was operated there appeared to be some leakage from the pasteurizer before pasteurization was complete. The plant foreman was found to be a carrier and was relieved of duty on April 16. Accordingly, he could not have been the cause of the cases during the second peak of the epidemic. It appeared that a portion of the raw milk coming to the plant was distributed without being pasteurized, since at certain times more raw milk was delivered than was run through the pasteurizer.

Additional Examples. In Anoka, Minn.,[79] a typhoid epidemic was traced to butter. All the patients patronized one grocer, and the butter came from a farm during the illness and convalescence of an unreported case of typhoid. At the time of the report between 35 and 40 cases, with 6 deaths, had occurred.

A cheese-borne outbreak of typhoid occurred in Alberta,[80] with 83 cases and 7 deaths. The outbreak covered a large area, and the cases had eaten green cheese made in a small plant from raw milk which came from 96 farms; 223 persons handled the milk and, of these, 19 gave histories of having had typhoid and 51 of contact with typhoid. A carrier was detected in the first group. *S. typhosa* cultures from 6 of the cases and from the carrier were of the same type.

Sources of *S. typhosa* in Milk. The sources from which *S. typhosa* enters milk vary widely. Importance of the various sources has changed with the more widespread use of pasteurization and the improvement in plant methods.

In 1927, Armstrong and Parran [2] summarized as follows the sources of the organism in 373 outbreaks for which the sources were known:

Sources from Which Milk Was Infected with Typhoid Organisms

Probable Source of Infection	Number of Outbreaks
Carrier (farm, distributing plant, etc.)	162
Active case (farm, distributing plant, etc.)	134
Exchange of bottles from homes with infection	37
Use of polluted water on utensils, etc.	28
Soilage of cows in polluted water	4
Miscellaneous (intermediate persons, etc.)	8

In modern milk plants pasteurization controls the dangers from carriers and cases on farms, more adequate bottle washing and sterilizing equipment reduces the danger from returned bottles, and better water supplies largely eliminate the chances of typhoid organisms getting into milk from water. Carriers and cases in the plants still are a hazard although modern milk plant operations minimize it.

Paratyphoid Fever

The spread of paratyphoid fever through dairy products shows the same general features as the spread of typhoid.

Example of Milk-borne Paratyphoid. An outbreak of paratyphoid affecting primarily infants and young children occurred in New Rochelle, N. Y., and 9 neighboring communities.[81] A milker at a dairy selling certified milk, who was found to be a carrier of *S. schottmuelleri*, was the source of infection. Each of the patients used the milk in question, and there were no similar cases among persons using any other milk. The clue to diagnosis of the outbreak was the isolation of *S. schottmuelleri* from the stool of 1 of the children during convalescence; the organism then was obtained from 3 other children. *S. schottmuelleri* was isolated from the stool of the carrier and also from the stool of his 4-yr-old child. The last raw certified milk from the dairy was sold on May 7, and the last case reported became ill on May 13.

Dysentery and Diarrhea

The general characters and methods of distribution of the various organisms responsible for dysentery and diarrhea suggest that these diseases can be spread through dairy products and a number of epidemics have been traced to them.

Septic Sore Throat

Although outbreaks of milk-borne septic sore throat had been recognized in Europe for many years, comparatively little attention was given them in the United States until 1912, when several serious epidemics occurred. Milk-borne septic sore throat is included in the group of diseases in which the infectious material comes primarily from man although sometimes the milk has been infected from abnormal udders which acquired the organisms from human sources. Many cases of septic sore throat are due to contact, but definite epidemics commonly include cases with more or less characteristic symptoms and sometimes have involved milk supplies. The outbreaks frequently have been extensive and, accordingly, attracted a great deal of attention. The mortality is low, with the deaths occurring principally among old people as a result of complications.

Examples of Milk-borne Septic Sore Throat. An epidemic of sore throat, involving more than 10,000 cases, occurred in Chicago.[82,83] Physicians attending the cases agreed that the epidemic was quite different from ordinary tonsillitis. After milk was suggested as a cause, investigation showed that most of the cases were consumers of milk from dairy X. Of 173 cases selected because of their severity and conformity to the epidemic type of throat infection, 147 (85%) were users of milk from dairy X at the time of illness; 15 (79%) of the 19 fatal cases used this milk. In the districts investigated, dairy X supplied about 10 or 12% of the milk. Of the households using milk from dairy X, 51% were affected as contrasted with 7.2% of those using other milk. At one hospital the 70 occupants of the children's wards passed through the epidemic without a case of sore throat, but among the nurses and internes there were many cases. All the milk for the hospital came from dairy X, but the milk for the children was delivered raw and pasteurized at the hospital; the remainder of the milk came from the usual pasteurized supply.

Sore throats of the epidemic type were present on 11 farms supplying dairy X, with a total of 29 cases. On 9 of these farms there also was mastitis among the cows. On one farm milk from a cow having typical mastitis of long duration contained streptococci, and streptococci also were obtained from a girl who had been ill with sore throat 5 weeks before. Both human and bovine cultures were fatal to guinea pigs and rabbits. The milk sold by dairy X was flash pasteurized, supposedly at a temperature of 160 F. Fluctuations in the temperature occurred, however, with a minimum of 130 F on several days. There

were pronounced failures of the pasteurization on certain days and explosive outbreaks of cases appeared to follow these dates rather closely. Final responsibility for the epidemic undoubtedly rested on inadequate pasteurization.

An epidemic[84] of septic sore throat, affecting 136 persons, occurred in Salmon Arm, B. C., a town having a population of about 900. The outbreak was confined to the town, with very few cases in the surrounding rural district. It was explosive in nature, lasted 1 month, and involved almost as many adults as children. Of the persons infected, 81% obtained their milk from the same dairy. Investigation revealed that an employee of the dairy had been ill with a severe sore throat some time previous to the epidemic; one of the cows developed mastitis subsequent to this, following an udder injury; and all persons having to do with handling of the milk on the farm were among those involved in the epidemic.

Example of Septic Sore Throat Due to Reconstituted Milk. Allen and Baer[85] reported an outbreak of septic sore throat due to milk reconstituted from powder; apparently it was contaminated in a mechanical cow at a naval station. About 10% of the complement of the station was ill. An inspection of the galley and food handlers was made early in the epidemic. Defects noted in the dairy room and preliminary case records made it seem probable that milk was the source of the infection. A rapid subsidence of the outbreak followed discontinuance of its use. The epidemiological evidence incriminated the general mess because 100% of the patients had eaten at the mess, whereas large groups of personnel at the station eating at other messes were not sick. Of the first 100 patients admitted to the sick list, all gave a history of drinking milk. No other food could be similarly incriminated. One of the 2 men assigned to work in the milk preparation room gave a history of having had a sore throat 2 weeks before the outbreak, and he had a recurrence during the epidemic. The study was considered to indicate a new source of danger to military personnel.

Sources of Causative Organisms in Milk-borne Septic Sore Throat. The probable sources of the causative organisms in 42 milk-borne septic sore throat epidemics were summarized by Armstrong and Parran;[2] cases on farms were involved in 16 epidemics, mastitis in cows in 3, cases on farms and mastitis in cows in 9, carriers on farms in 2, and the source was undetermined in 12. The more recent outbreaks also emphasize the importance of cases, carriers, and mastitis in cows as sources of the organisms involved.

Ramsey [86] reported data on 13 epidemics of septic sore throat, due to raw milk, which were investigated in detail at the time of occurrence or shortly after. In 7 instances human illness had occurred on the incriminated farm prior to the outbreak; in 4 of these there were sore throats and in 3 draining wounds, or hand infections, in dairy workers. Human cases occurred on the suspected farm in 4 epidemics while they were in progress but not previously, as far as could be determined. In only 2 epidemics was it impossible to find evidence of illness among persons on the farms involved. Ramsey interpreted these results as indicating that, when septic sore throat occurs, the original source of the infection is more likely to be an ill person than a healthy carrier.

Scarlet Fever

Although scarlet fever is contracted principally through direct contact, the causative organism occasionally gets into dairy products and then may reach susceptible persons.

Examples of Milk-borne Scarlet Fever. Welch and Mickle [87] reported an outbreak of scarlet fever and septic sore throat that epidemiological evidence indicated was due to heavy infection of a milk supply. Of 5,000 qt delivered in the area, 240 came from the dairy involved; 68 of the 100 cases were in families using the infected milk, and most of the others clearly were accounted for by contact infections. Of 70 families residing in the part of town where the outbreak occurred, 32 used the infected supply and 18 had 44 cases of scarlet fever and septic sore throat. The 14 families taking infected milk, but having no cases, included 4 that reported scarlet fever previously, 7 that consisted of 14 adults and 1 child, and 3 that scalded their milk. The organisms isolated from clinical cases of scarlet fever and sore throat showed the biochemical and morphologic characteristics of the scarlet fever organism. Two milk handlers operating the dairy connected with the outbreak on epidemiological evidence harbored in their throats organisms identical in every respect with those isolated from clinical cases.

An epidemic of approximately 500 cases of scarlet fever was traced to use of raw milk from an infected cow.[88] The source of the outbreak was suspected early in the epidemic and sale of raw milk from the dairy concerned was stopped; this action was followed by rapid decline in the number of cases. Investigation revealed that the suspected animal had been added to the herd at the dairy farm a few days previous to the beginning of the outbreak. Immediately prior to

that, the cow had been on a farm where 3 cases of scarlet fever had occurred, 1 in a boy who had milked the cow; the evidence indicated the animal had acquired the infection there.

Diphtheria

Diphtheria is contracted primarily through contact with human sources, either cases or carriers but, occasionally, eliminated organisms encounter conditions that permit their survival for extended periods and a number of epidemics have been traced to dairy products.

Example of Milk-borne Diphtheria. Hedrich [89] reported an instance in which diphtheria appeared to be spread through an infection on the outsides of milk bottles. About 15 positive throat swabs were received in a city laboratory, 1 or 2 a day over a period of 10 days. Nearly all the patients were under 6 yr of age, which suggested a milk-borne epidemic. Investigation showed that most of the involved families patronized one milk dealer who served about 1,500 customers with pasteurized milk through 5 wagons. The small number of sporadic cases among a large number of customers was soon explained. Among the milk handlers there was a person whose throat contained the diphtheria organism. Part of his work was to inspect bottles for cracks and he easily could have infected his hand and then the outsides of the bottles when he lifted them by the neck. The diphtheria organism presumably got into the milk when it was poured from the bottles. The epidemic was stopped by quarantining the carrier.

Poliomyelitis

Epidemiological evidence indicates that poliomyelitis occasionally has been contracted through milk.

Examples of Milk-borne Poliomyelitis. Aycock [90] reported an epidemic of poliomyelitis that occurred in Broadstairs, England. The outbreak started and subsided suddenly. Multiple cases occurred in private boarding schools having little communication with the town or with each other. In the 5 yr previous to the outbreak only 2 cases of poliomyelitis had been reported in Broadstairs. Practically all the cases were supplied with milk from dealer A. Although 3 boarding schools in which cases occurred were supplied by dealer F, these may have obtained milk indirectly from dealer A, since for a period he supplied 44 qt daily to dealer F. Four persons who left Broadstairs shortly before the occurrence of the epidemic developed the disease, and while in Broadstairs each of them had lived in a house supplied by dealer A. Of the total 75 cases, 50 were known to have used milk

from dealer A, and 19 others (in private boarding schools) probably received this milk.

Lipari [91] obtained epidemiological evidence supporting the view that an epidemic of poliomyelitis involving 23 paralytic and 17 nonparalytic cases was spread by milk. Unhygienic conditions prevailed at the dairy supplying the milk and the son of the operator had been suffering from poliomyelitis. A significantly higher consumption rate of the milk was found among the patients than among 247 unidentified controls in the area.

Cholera

A number of milk-borne epidemics of cholera have been recorded, and such outbreaks are to be expected from the frequent distribution of the disease through water. In certain countries in which cholera is common, the practice of boiling milk probably prevents many infections.

Examples of Milk-borne Cholera. During World War I the British Army operating in the Near East experienced acute epidemics of cholera in 2 hospitals.[92] In one hospital there were 35 cases with 14 deaths and in the other 19 cases with 10 deaths. In both instances the infection was carried by the milk supply. The milk commonly was sterilized before it was given to the patients, but often it arrived late and the sterilization was not always supervised.

Intestinal Disturbances in Children

Intestinal disturbances in children may be responsible for illness and deaths, especially among young children. These conditions appear to be related, in part, to the food, since various data show that, at one time, cases were much more common among artificially fed infants than among those breast fed.

Much of the early work on milk improvement was stimulated by illness among bottle-fed infants, and better milk supplies have been a factor in its decrease. Often excessive numbers of organisms of various types in milk appear to have been involved, rather than specific types, but in certain instances definite species presumably were responsible. At present, satisfactory milk supplies and the attention given the feeding of infants from the standpoint of the modification of the milk and its heat treatment largely have eliminated the bacteriological condition of milk as a factor in intestinal disturbances, except where carelessness prevails.

POISONING FROM DAIRY PRODUCTS

Various dairy products are included in the large group of food materials that may cause poisoning. In some instances they have been considered responsible for poisoning on very meager evidence, but in other cases careful investigations have established their relationship to the condition. A number of ways in which dairy products may acquire poisonous properties have been suggested; these include (1) conditions involving the producing animal, such as elimination of drugs, substances contained in the feed, and substances resulting from disease in the animal, and (2) conditions developing after production of the milk, such as absorption of metals and action of micro-organisms.

The observations of Burke *et al.*[93] are of interest in connection with absorption of metals. After deaths among swine that were attributed to buttermilk held in a galvanized iron tank, the investigators carried out studies which showed buttermilk dissolved zinc from surfaces of galvanized vessels in proportion to the acidity and period of exposure. Under the conditions of the experiment, souring of milk in galvanized containers had no deleterious effect on growth and reproduction in rats and swine. Small amounts of zinc lactate also had no marked effect, but a relatively large amount of zinc carbonate (1% or more) appeared to retard growth and reproduction in rats.

Examples of Poisoning from Dairy Products. Ex. 1. Macaulay[94] reported an outbreak of cheese poisoning involving Canadian cheddar type. Because of its rather distinctive appearance, the cases investigated definitely could be traced to it. Out of a total of 135 individuals who were known to have eaten the cheese, 126 included in 43 families were poisoned. In every case the symptoms began a short time after eating the cheese, and no other article of food was common to all the cases; the incubation periods varied from 3.5 to 11 hr. Three persons, after recovery from the initial attack and not suspecting the cheese, ate it a second time and experienced another attack. There were no deaths. The cheese appeared perfectly wholesome. A few small holes, apparently due to gas, were noted in pieces of it and did not appear in other cheese of the same lot. Feeding trials with mice gave irregular results, but in some instances symptoms of poisoning were noted. The one trial with a human subject yielded definite poisoning. No organism known to be associated with food poisoning was found in the cheese, or in specimens from cases, and mineral

poisons could not be detected. The outbreak was considered almost certainly to be due to a bacterial toxin.

Ex. 2. Homemade cottage cheese, which had caused the death of 3 persons who had eaten it, was studied by Nevin and Mann.[95] An anaerobic organism was isolated that killed mice, guinea pigs, and rabbits, and these animals also were killed by filtrates of cultures. By repeated injections of heated toxin, an antitoxin was obtained from a rabbit which protected a guinea pig against 100 times the minimum lethal dose of toxin. The organism was identified as *Clostridium botulinum.* Inoculation of the toxin into a kitten gave the typical picture of botulism.

Ex. 3. Weed *et al.*[96] presented data indicating that 3 patients developed food intoxication after drinking milk of a goat suffering from acute suppurative mastitis due to *M. pyogenes* var. *aureus.* Two of the patients (3 and 4 yr of age) died within 24 hr after each drank ¼ pt of the milk. Cooked milk did not cause illness. When the milk was drawn, nothing unusual was noted except that the yield was lower than normal. The goat died within 24 hr. The only significant findings post-mortem were limited to the udder, one side of which was swollen, red, and firm. An incision into the swollen side revealed marked congestion with numerous dilated lactiferous ducts containing a fluid suggestive of bloody milk. Material from the udder post-mortem reproduced the symptoms in kittens. There was a history of a scratch on a teat 2 weeks earlier.

Ex. 4. Tucker *et al.*[97] studied an outbreak of food infection involving 250 cases in Tennessee, 100 cases in Illinois, and 34 cases in Kentucky. All the patients had eaten colby cheese 24 to 48 hr prior to the onset of illness. In many families persons became ill after eating cheese, and persons not eating cheese remained well. The cheese was on the market soon after manufacture. Examination of a sample of cheese resulted in isolation of *Salmonella typhimurium.* At 43 to 48 F the organism remained viable in a 6-lb piece of cheese for 302 days, examinations being made at monthly intervals.

Other Outbreaks. The many outbreaks of poisoning due to dairy products vary greatly in the conditions and products involved. An outbreak [98] involving some 6,000 cases was traced to camembert cheese infected with *Salmonella sp.* (Type Bareilly). Another [99] affecting 252 persons was caused by raw milk from a farm on which a cow was shedding S. *typhimurium* in her milk and feces; the number of cases fell rapidly when the milk was pasteurized. In an outbreak [100] of

gastroenteritis affecting about 50 persons, the causal organism was isolated from the feces of some of the patients and identified as *Salmonella sp.* (Type Dublin); it also was isolated from individual quarter samples from 1 cow.

Of 435 outbreaks [101] (including family outbreaks) of food poisoning in England and Wales during 1950 and in which the vehicle of transmission was noted, 12 were caused by liquid milk, 1 by tinned milk, 3 by dried milk, 5 by cheese, and 15 by ice cream. Four milk-borne outbreaks were due to *S. typhimurium* and 5 to micrococci.

Identification of Responsible Organisms. In studies on food intoxications and infections, the isolation and identification of the responsible organisms frequently are very desirable. To obtain proof that an organism which appears to be responsible for an outbreak actually was involved often is difficult. The studies along this line have resulted in the development of procedures that simplify the problem.[102]

GROWTH AND LONGEVITY OF PATHOGENS IN DAIRY PRODUCTS

Milk. The nutrients provided by milk are adequate for the growth of various pathogens but multiplication under practical conditions is influenced by various factors. Following earlier investigations,[103] Wahby and Sherman [104] studied the growth in milk of 3 species of the typhoid-dysentery group, 4 of the paratyphoid group, and *Br. abortus, C. diphtheriae, V. comma, S. pyogenes,* and *S. agalactiae.* The trials were conducted in sterilized skim milk to simplify the counting. At 86 F all the organisms grew rapidly; at 59 F all grew except *Br. abortus* and *V. comma* although the growth of *C. diphtheriae* and one of the dysentery organisms was feeble; at 50 F the 4 paratyphoid species grew, and *S. typhosa* sometimes showed very slight growth after several days; at 45.5 F and at 41 F none of the organisms grew. Pullinger and Kemp [105] found that various species of *Salmonella* multiplied readily in fresh raw milk at 59 F and also in commercial grades of raw and heat-treated milk.

In trials by Kuzdas and Morse,[106] raw milk heavily inoculated with *Br. abortus* and incubated at 77 and 98.6 F still contained large numbers after 24 hr but none after 48; at −40 F the count still was high after 800 days.

Although *M. tuberculosis* develops well on sterile milk held at favorable temperatures, multiplication probably never occurs in milk under practical conditions because the organism has a rather definite growth

temperature, especially when it has just come from a lesion, and also because growth starts slowly.

Investigations have shown that with lactic acid in milk *S. typhosa* gradually is killed. Extent of the contamination influences the time of survival.[107] Acid-tolerant strains apparently multiply in amounts of acid which destroy the majority of cells and products of bacterial growth other than acid may play a part in the destruction.[108] Wilson and Tanner[109] found that *S. typhosa*, 2 *Salmonella* species, and 2 *Shigella* species were destroyed more rapidly when lactic acid was added to milk than when natural fermentation occurred and that slow increases in acid permit development of acid-tolerant species if they are present. The authors noted that in naturally infected milk, the acidity developed is not sufficient to destroy, in a reasonable period, the pathogens tested; with 0.95 to 1.15% acid (*p*H 4.2 to 3.9), the organisms survived 1 day to 7 weeks and, with 0.50 to 0.86% (*p*H 5.0 to 4.3), they persisted 2 to 9 weeks.

Kästli and Binz[110] adjusted the *p*H values of sterile milk, added *Br. abortus*, and stored it at 100.4 F. The organism was killed in 9 hr at *p*H 4.03, in 9 to 14 hr at *p*H 4.27, and in 24 hr at *p*H 4.6. The bactericidal effect of lactic acid appeared to be higher than that of hydrochloric acid. Nagel[111] inoculated raw buttermilk with *S. typhosa*, *S. schottmulleri*, and *E. coli* and incubated it at 39.2, 71.6, and 98.6 F. The samples incubated at 98.6 F were negative for *S. typhosa* and *S. schottmulleri* after 6 hr, but the latter organism and *E. coli* still could be detected in samples incubated at 71.6 F after 25 hr.

Ice Cream. In ice cream stored in a hardening room that fluctuated from −8 to 8 F, Prucha and Brannon[112] found that there was a continual decrease in the number of inoculated typhoid organisms but that some still were alive after 2 yr and 4 months, when the examinations were terminated. Wallace[113] found various pathogens, including *Salmonella*, *Brucella*, and *Mycobacterium* types, survived in ice cream for several years.

Butter. The viability of pathogens in butter was investigated by Berry;[114] it was stored mainly at ice-box temperature, which usually was 50.9 F, although a few samples were held at room temperature. After inoculation of the butter there was an immediate decline in the numbers of organisms in most cases. The organisms studied showed wide variations in their viability in butter. *S. schottmulleri* survived 212 days. Strains of *S. typhosa* varied considerably; 1 strain survived only 22 days, or less, another survived 63 days, and still another survived at least 110 days. *S. paratyphi* survived 117 days; the

dysentery organisms disappeared in 7 to 18 days; and *S. pyogenes* survived a maximum of 17 days. Berry found that in milk at ice-box temperature *S. schottmulleri* survived 324 days, *S. paratyphi* 107 days, a strain of *S. typhosa* 290 days, and *S. pyogenes* 17 days. Washburn [115] noted that in an ice chest *S. typhosa* lived 151 days in butter and 43 days in milk; it was actively motile in milk for 20 days. In connection with the study of a butter-borne diphtheria outbreak, the organism was found in butter after 1 month.[116]

Bryan and Bryan[117] infected a cow with 2 streptococci and 2 micrococci, each in a separate quarter, and after collecting the cream added a broth culture of *Br. abortus* and made butter from it. At about 45 F *Br. abortus* remained viable 4 months in raw sweet-cream butter and 3 months in raw ripened-cream butter. *S. agalactiae, S. pyogenes,* and both hemolytic and nonhemolytic *M. pyogenes* var. *aureus* persisted in raw sweet-cream or raw ripened-cream butter for 6 months. Results were the same with 2% salt in the butter as with no salt. Butter made from the cream after pasteurization gave only negative results.

Trials carried out when considerable human tuberculosis due to the bovine organism still was occurring in the United States showed that the organism lived in butter for extended periods, often several months. Survival was noted at 60 to 68 F,[118, 119] in an ice box,[120] and under commercial storage.[119, 121] Tuberculosis was most severe in inoculated guinea pigs with the butter held at the lowest temperatures.[119] The disease was produced in guinea pigs by feeding but not as regularly as with inoculation.[121]

Cheese. Wade and Shere [122] reported a cheese-borne typhoid epidemic in which the development of cases indicated *S. typhosa* lived in the cheese approximately 63 days; the cheese was made from milk which did not curdle satisfactorily. In 18 experimental cheese made from milk infected with *S. typhosa,* the organism usually lived from 0 to 8 days, averaging about 4 days, but with 2 cheese the organism was recovered on every test until no more material was available, which was on the thirty-fourth and thirty-sixth days after manufacture. Since these 2 cheese were made from the same lot of milk, longevity of the organism in the cheese may have been influenced by the flora of the cheese.

Campbell and Gibbard [123] studied survival of *S. typhosa* in small commercial-size cheddar cheese. There was a significant difference in the viability in cheese stored at 40 to 42 and 58 to 60 F; both lots of cheese were held at 58 to 60 F for 14 days before being waxed for

holding. In the cheese held at 58 to 60 F, most of the *S. typhosa* cells were dead after 3 months, whereas the cheese held at 40 to 42 F contained viable *S. typhosa* for at least 6 months and in 7 out of 10 cases for 10 or more months. There appeared to be no difference in the longevity of the organisms when small or large inocula were used, but a difference in resistance of different types of the organism was suggested.

Gilman *et al.*[124] studied the survival of *Br. abortus* in cheese. Cheddar cheese was made from milk produced by a herd highly infected with brucellosis. The milk contained about 500 viable *Br. abortus* organisms per ml and a freshly isolated culture was added to make about 1,000. The organism survived in the cheese for 6 months at 40 F, but only negative results were obtained after 1 yr. With milk produced by cows eliminating large numbers of *Br. abortus*, there were 700 to 800 of the organisms per ml. All the cheese were positive after 3 months, some were negative after 6 months, and all were negative after 1 yr. Two vats of limburger cheese milk in New York were positive to *Br. abortus*, but the curds were negative and the cheese were negative when first examined after 57 days. With 34 vats of commercial cheddar cheese milk in New York and Wisconsin, 9 lots of milk and 8 lots of curd were positive to *Br. abortus*. Two cheese were lost and the other 7 from the positive lots of milk were all free from viable *Br. abortus* when first examined after 41 to 84 days at 34 to 36.9 F.

Early studies indicated that *M. tuberculosis* lives in cheeses for significant periods.[125] Mohler *et al.*[126] reported that infected cheddar cheese, obtained by adding the organism to the milk, produced generalized tuberculosis in inoculated guinea pigs when it was 220 days old. The more recent studies have emphasized the effect of the type of cheese on survival. Kästli and Binz [127] made cheeses from milk of cows having tuberculosis. Results indicated that in emmenthaler and gruyère cheeses the organism was not viable after 5 to 30 days, in tilsit cheese it was detected after 232 and 305 days, and in camembert and munster cheeses it was present after 47 days (final examination). Cottage cheese was made by Loncin and Geairain [128] from milk contaminated with milk from a tuberculous cow. Tests for *M. tuberculosis* were positive up to 14 days, but after 17 days the results were negative or doubtful. The cheese began to undergo alkaline putrefaction after 2 weeks. It was suggested that putrefactive organisms produce materials with antibiotic properties. Seelemann *et al.*[129] used milk containing 0.2% of milk from a tuberculous cow and

made it into camembert cheese, either raw or heat treated. *M. tuberculosis* was found in the raw milk cheese up to 3 months at temperatures between 59 and 14 F. When the milk had been heated 30 min at 132.8 F, 40 sec at 149 F, or 10 sec at 158 F, the organism was detected in the fresh cheese but not in that ripened 20 days at 59 F. With higher heated milk the organism was not found.

General Considerations. The data available indicate that under certain conditions pathogens may live in dairy products for long periods, with various factors influencing the survival time, and also that holding products containing pathogens may not make them safe. Accordingly, attempts to ensure the safety of such a product as raw milk cheese by holding for a given period is open to question. This is especially true when a low holding temperature appears to extend the period an organism will survive in such a product as cheese and requirements covering the holding commonly ignore the temperature.

The apparent increase in the number of cheese-borne outbreaks of typhoid during recent years has emphasized the danger of using raw milk for manufacturing purposes. The logical solution of the problem is pasteurization of the milk for cheese; this is in agreement with the widespread use of heat treatment with other dairy products.

LABORATORY EXAMINATIONS OF DAIRY PLANT EMPLOYEES

The importance of human cases and carriers of various diseases as sources of pathogens in dairy products indicates the need for the examination of dairy plant employees in order to exclude persons who may infect supplies of such products. In connection with grade A pasteurized milk, the U. S. Public Health Service Milk Ordinance and Code treats the problem in detail. These requirements also are advisable for employees of plants producing other dairy products. In some cases the health examinations used are incomplete, supply only inadequate information, and do not give the desired consumer protection.

It appears that many health authorities feel that application of the health-examination principle is limited, for practical purposes, to examinations for active tuberculosis, diphtheria, and typhoid and paratyphoid fevers.

PREVENTING DISSEMINATION OF DISEASES THROUGH DAIRY PRODUCTS

The methods of preventing spread of diseases through dairy products are (1) prevention of entrance of pathogens, and (2) destruction of any pathogens that may be present.

Consideration of the sources from which pathogens get into dairy products shows that the thorough control of all of them is a tremendous undertaking that begins with the health of the producing animals and ends only when the dairy product is used. Perhaps the greatest problem is in connection with the persons coming in contact with dairy products since any one of them suddenly may be capable of eliminating pathogens. Attempts that are made to control all sources of pathogens in milk add greatly to the cost, and such specially produced milk is not available in most areas. Moreover, some of the attempts to prevent the entrance of pathogens into milk have not been successful and cases of disease have resulted. Epidemics of various diseases have been spread through milk produced under conditions that were intended to be excellent. On the other hand, careful supervision of milk production and milk processing undoubtedly has prevented many epidemics.

Destruction of the pathogens that may be present in milk and cream is carried out most readily by proper pasteurization, and this controls the dangers up to the time of the treatment. There is the possibility of infection of milk and cream after pasteurization, but this is small compared to the possibility of infection before the milk and cream reach the pasteurization plant and can be controlled by proper methods. The pasteurization must be efficient if the anticipated protection is to be obtained; a number of epidemics have been traced to milk that was improperly heated because of some defect in the equipment or its operation.

Accomplishments of Milk Control. Where well-organized attempts have been made to control milk supplies, a great deal has been accomplished. This is illustrated by the following data reported by Feemster [130] on milk-borne diseases in Massachusetts; presumably, during the early part of the period covered, recognition of milk-borne outbreaks was very incomplete.

Decrease in Milk-borne Outbreaks of Disease in Massachusetts

Period	Number of Outbreaks	Period	Number of Outbreaks
1886–1890	3	1921–1925	17
1891–1895	4	1926–1930	14
1896–1900	15	1931–1935	7
1901–1905	20	1936–1940	4
1906–1910	28	1941–1945	3
1911–1915	45	1946–1950	2
1916–1920	32		

Data on the milk-borne disease outbreaks in the United States are compiled each year by federal agencies from reports submitted by state and local health officials; a summary of the results is as follows:

Total Reported Outbreaks of Milk-borne Diseases in the United States

Year	Number of Outbreaks	Year	Number of Outbreaks	Year	Number of Outbreaks
1923	23	1934	42	1945	29
1924	44	1935	43	1946	19
1925	44	1936	42	1947	22
1926	68	1937	43	1948	17
1927	36	1938	42	1949	15
1928	46	1939	41	1950	10
1929	51	1940	43	1951	12
1930	48	1941	37	1952	6
1931	34	1942	45	1953	4
1932	33	1943	40	1954	9
1933	42	1944	41	1955	3

The data show that in the United States as a whole outbreaks of milk-borne diseases still occur. Undoubtedly, recognition of milk-borne outbreaks has been more complete recently than it was formerly, but it has been reasonably complete for a rather long period. Many of the outbreaks occur in areas where there is only inadequate milk control. Frequently, a raw milk supply is responsible but pasteurized milk also is involved because of inadequate heat treatment of a part or all of the milk or contamination of properly pasteurized milk. The most significant changes in the types of outbreaks occurring have been a decrease in outbreaks of typhoid and an increase in outbreaks of food poisoning and gastroenteritis.

The general situation indicates the need for greater emphasis on the control of milk-borne outbreaks in certain sections of the United

States. With large population groups showing no such outbreaks over extended periods, the possibility of practically complete control has been demonstrated clearly. With sufficient attention to supervision of milk supplies, there should be no milk-borne outbreaks of disease.

REFERENCES

1. Swithinbank, H., and Newman, G. *Bacteriology of Milk*, 260, 1903. John Murray, London.
2. Supp. 62, *Public Health Repts.* (1927).
3. *Berlin. klin. Wochschr.*, **19**, 221 (1882).
4. *Trans. Assoc. Am. Physicians*, **11**, 75 (1896).
5. *J. Exptl. Med.*, **3**, 451 (1898).
6. *J. Med. Research*, **25**, 313 (1911).
7. *J. Med. Research*, **27**, 109 (1912).
8. *J. Hyg.*, **47**, 337 (1949).
9. *Can. Public Health J.*, **25**, 13 (1934).
10. *Am. J. Public Health*, **17**, 491 (1927).
11. *Milchwissenschaft*, **6**, 355 (1951).
12. *Dairy Inds.*, **18**, 210 (1953).
13. *Standard Methods for the Examination of Dairy Products* (Last ed.). American Public Health Association, Inc., New York, N. Y.
14. *Ill. Agr. Expt. Sta. Bull.* 149 (1911).
15. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 299 (1911).
16. *Final Rept. Great Britain Royal Comm. Human Animal Tub.* **3**, 79 (1911).
17. *Wis. Agr. Expt. Sta. Bull.* 143 (1907).
18. *Am. J. Public Health*, **42**, 1253 (1952).
19. *Practitioner*, **39**, 161 (1887).
20. *U. S. Public Health Marine Hospital Service Hyg. Lab. Bull.* 56, 201 (1909).
21. *Public Health Repts.*, **37**, 2895 (1922).
22. *Z. Tiermed.*, **1**, 241 (1897).
23. *J. Infectious Diseases*, **22**, 580 (1918).
24. *U. S. Dept. Agr. Bur. Animal Ind., 28th Ann. Rept.*, 139 (1911).
25. *U. S. Dept. Agr. Bur. Animal Ind., 28th Ann. Rept.*, 147 (1911).
26. *Can. Public Health J.*, **25**, 5 (1934).
27. *Cornell Vet.*, **22**, 212 (1932).
28. *Public Health Repts.*, **45**, 2433 (1930).
29. *J. Infectious Diseases*, **85**, 213 (1949).
30. *Public Health Repts.*, **64**, 1021 (1949).
31. *Am. J. Vet. Research*, **12**, 165 (1951).
32. *J. Am. Med. Assoc.*, **102**, 1670 (1934).
33. *Conn. State Dept. Health 50th Rept.*, 170 (1935).
34. *J. Milk and Food Technol.*, **16**, 263 (1953).
35. *Public Health Repts.*, **64**, 41 (1949).
36. *J. Am. Med. Assoc.*, **145**, 301 (1951).
37. *J. Am. Med. Assoc.*, **145**, 306 (1951).
38. *Am. J. Hyg.*, **54**, 1 (1951).
39. *Am. J. Public Health*, **39**, 485 (1949).
40. *Public Health Repts.*, **63**, 1611 (1948).
41. *Am. J. Public Health*, **39**, 478 (1949).
42. *J. Am. Vet. Med. Assoc.*, **118**, 170 (1951).
43. *Am. J. Hyg.*, **54**, 35 (1951).
44. *Am. J. Hyg.*, **54**, 44 (1951).

45. *Public Health Repts.*, **65**, 395 (1950).
46. *Public Health Repts.*, **66**, 1685 (1951).
47. *Intern. Assoc. Dairy and Milk Inspectors, 13th Ann. Rept.*, 166 (1924).
48. *Johns Hopkins Hosp. Bull.*, **26**, 351 (1915).
49. *J. Am. Med. Assoc.*, **66**, 947 (1916).
50. *Wis. Agr. Expt. Sta. Research Bull.* 81 (1927).
51. *J. Comp. Pathol. Therap.*, **54**, 120 (1944).
52. *J. Comp. Pathol. Therap.*, **22**, 148 (1909).
53. *N. Y. State J. Med.*, **19**, 138 (1919).
54. *La. Agr. Expt. Sta., 33rd Ann. Rept.*, 17 (1921).
55. *Vet. J.*, **70**, 132 (1914).
56. *Australian Vet. J.*, **6**, 2 (1930).
57. Chapin, C. V. *The Sources and Modes of Infection*, 2nd ed., 366, 1912. John Wiley and Sons, Inc., New York, N. Y.
58. *J. Med. Vet. Zootech.*, **56**, 721 (1905).
59. *J. Lab. Clin. Med.*, **15**, 460 (1930).
60. *Intern. Assoc. Dairy and Milk Inspectors, 22nd Ann. Rept.*, 120 (1933).
61. *N. C. Agr. Expt. Sta. Tech. Bull.* 15 (1918).
62. *U. S. Dept. Agr. Bull.*, 1391 (1926).
63. *J. Agr. Research*, **9**, 397 (1917).
64. *J. Agr. Research*, **11**, 699 (1917).
65. *Med. Record*, **92**, 428 (1917).
66. *J. Infectious Diseases*, **24**, 231 (1919).
67. *J. Agr. Research*, **35**, 547 (1927).
68. *J. Agr. Research*, **40**, 649 (1930).
69. *J. Am. Med. Assoc.*, **91**, 234 (1928).
70. *Milk Plant Monthly*, **37** (12), 34 (1948).
71. *Pa. Agr. Expt. Sta. Bull.*, 525 (1950).
72. *Vet. Record*, **63**, 141 (1951).
73. *Edinburgh Coll. Agr. Tech. Bull.* 2 (1947).
74. *Schweiz. Z. allgem. Pathol. Bakteriol.*, **14**, 545 (1951).
75. *Milk Plant Monthly*, **38** (10), 42 (1949).
76. *Brit. Vet. J.*, **106**, 181 (1950).
77. *Am. J. Public Health*, **19**, 257 (1929).
78. *Am. J. Public Health*, **17**, 783 (1927).
79. *Minn. State Bd. Health 4th Bien. Rept.*, 240 (1913).
80. *Can. J. Public Health*, **35**, 431 (1944).
81. *J. Am. Med. Assoc.*, **84**, 251 (1925).
82. *J. Am. Med. Assoc.*, **58**, 1848 (1912).
83. *J. Am. Med. Assoc.*, **58**, 1852 (1912).
84. *Can. J. Public Health*, **36**, 419 (1945).
85. *J. Am. Med. Assoc.*, **124**, 1191 (1944).
86. *Intern. Assoc. Dairy and Milk Inspectors, 24th Ann. Rept.*, 163 (1935).
87. *Am. J. Hyg.*, **17**, 229 (1933).
88. *Am. J. Public Health*, **27**, 246 (1937).
89. *Am. J. Public Health*, **8**, 457 (1918).
90. *Am. J. Hyg.*, **7**, 791 (1927).
91. *N. Y. State J. Med.*, **51**, 362 (1951).
92. *Med. Services, Diseases of the War*, **1**, 119 (1922).
93. *J. Dairy Sci.*, **11**, 79 (1928).
94. *Lancet*, **203**, 1012 (1922).
95. *N. Y. State Dept. Health, 36th Ann. Rept.*, 3, 306 (1915).
96. *Am. J. Public Health*, **33**, 1314 (1943).
97. *J. Am. Med. Assoc.*, **131**, 1119 (1946).

98. *Deut. med. Wochschr.*, **78**, 1412 (1953).
99. *Monthly Bull. Ministry Health Lab.*, 13, 90 (1954).
100. *Med. Officer*, **82**, 267 (1949).
101. *Monthly Bull. Ministry Health Lab.*, 10, 228 (1951).
102. Dack, G. M. *Food Poisoning* (3rd ed.), 1956. University of Chicago Press, Chicago, Ill.
103. *Mass. State Bd. Health, 39th Ann. Rept.*, 417 (1907).
104. *7th Ann. Rept. N. Y. State Assoc. Dairy and Milk Inspectors*, 83 (1933).
105. *J. Hyg.*, **38**, 587 (1938).
106. *Cornell Vet.*, **44**, 216 (1954).
107. *Bur. Labs. City N. Y. Coll. Studies*, **8**, 313 (1914–1915).
108. *J. Infectious Diseases*, **16**, 479 (1915).
109. *Food Research*, **10**, 122 (1945).
110. *Schweiz. Milchztg.*, **74**, 8 (1948).
111. *Med. Klin.* (*Munich*), **45**, 739 (1950).
112. *J. Bacteriol.*, **11**, 27 (1926).
113. *J. Dairy Sci.*, **21**, 35 (1938).
114. *J. Prevent. Med.*, **1**, 429 (1927).
115. *U. S. Dept. Agr. Bur. Animal Ind., 25th Ann. Rept.*, 297 (1908).
116. *Minn. State Bd. Health 3rd Bien. Rept.*, 203 (1911).
117. *J. Milk Technol.*, **7**, 65 (1944).
118. *U. S. Dept. Agr. Bur. Animal Ind. Circ.* 153 (1910)
119. *Ill. Agr. Expt. Sta. Bull.* 161 (1912).
120. *U. S. Dept. Agr. Yearbook*, 431 (1895).
121. *U. S. Dept. Agr. Bur. Animal Ind., 26th Ann. Rept.*, 179 (1909).
122. *Am. J. Public Health*, **18**, 1480 (1928).
123. *Can. J. Public Health*, **35**, 158 (1944).
124. *J. Dairy Sci.*, **29**, 71 (1946).
125. *U. S. Dept. Agr. Bur. Animal Ind., 19th Ann. Rept.*, 217 (1902).
126. *U. S. Dept. Agr. Bur. Animal Ind., 26th Ann. Rept.*, 187 (1909).
127. *Milchwissenschaft*, **4**, 391 (1949).
128. *Lait*, **30**, 608 (1950).
129. *Milchwissenschaft*, **9**, 72 (1954).
130. *Am. J. Public Health*, **41**, 1275 (1951).

Milk Enzymes

10

Milk normally contains enzymes, the more important being lipase, phosphatase, protease, peroxidase, catalase, and oxidase. The milk enzymes require consideration in connection with the bacteria in dairy products because of the similarity of their activities and certain bacterial changes.

Differentiation of Enzymatic and Bacterial Changes. In the study of certain changes due to milk enzymes, a reagent, such as sugar, chloroform, formaldehyde, toluene, ether, etc., sometimes is used to prevent action of organisms. With this procedure it is advisable to examine, both culturally and microscopically, the material under investigation to be certain that organisms are not developing. The importance of this was shown through the isolation by Babcock and Russell [1] of an organism that possessed a definite ether tolerance; the character was lost during a series of transfers on agar.

The changes produced by enzymes and by bacteria differ in rate. With enzymes the active agent does not increase and, accordingly, the rate of change does not; it tends to fall off with time, whereas with bacteria the active mass increases and, as a result, the rate of change does also until conditions become unfavorable for the organisms. The activity of enzymes is dependent upon the *p*H of the medium; bacteria sometimes influence the action of enzymes through their effect on the *p*H.

LIPASE

Raw milk contains a group of enzymes which hydrolyze milk fat with liberation of free fatty acids. Evidence of more than one lipase in milk is based on the presence of formaldehyde-resistant and formaldehyde-sensitive enzymes,[2,3] as well as enzymes with different *p*H optima.[4] Lipase in milk is associated with the serum and, accordingly, acts at the surfaces of fat globules. Pfeffer *et al.*[5] found that, when milk was separated, greater lipolytic activity was associated with the skim milk than with the cream. As the fat content of cream was increased, the lipase activity decreased. Separator slime had about three times the lipolytic activity of the milk from which it came. Ordinarily, lipase activity is objectionable in a dairy product, but under certain conditions, notably in the ripening of blue-veined and Italian-type cheeses and in the preparation of certain milk derivatives, it is desirable.

Methods of Estimation. Various methods and modifications have been used to detect lipase activity in dairy products.

Organoleptic Examination. Taste and smell are the only means of detecting certain defects associated with dairy products, and the method has been used to detect lipolysis. The fatty acids from butyric through myristic are of most importance from the standpoint of flavor and odor;[6] butyric acid is of special significance because of its conspicuous odor although it makes up only a small part of the total fatty acids of milk fat. Flavors of dairy products due to fat hydrolysis have been called rancid, lipolytic, wintery, bitter, goaty, unclean, etc.

Increase in Titratable Acidity. In some of the early studies, milk or cream was titrated with alkali initially and again after a period of incubation, the increase being attributed to freed fatty acids. Several methods may be used to prevent an increase in acid due to microorganisms during extended incubation, such as holding at a low temperature or addition of a preservative. Rice and Markley[7] used as a substrate a high-fat cream containing sufficient sucrose to saturate it and any water included with the material to be tested; the mixture of cream and sugar was boiled to facilitate solution of the sugar and to destroy enzymes in the cream. In some cases the milk or cream to be tested for lipase activity, or that used in the substrate, is homogenized in order to increase the fat surface and thus facilitate lipase action. Titration methods measure the water-soluble fatty acids and generally lack sensitivity because of small titration values.[8]

Titration of Distillates from a Substrate. Rancid milk, cream, and other materials have been steam distilled and the volatile acids in the distillate estimated by titration.[3] Recovery of the most volatile acid (butyric) by steam distillation is rather low without extended distillation.[9]

Fat Acidity. Lipase activity has been measured by titrating the free acids associated with the fat.[2,10,11] Milk, cream, or other fat-containing substrate is held for a time under suitable conditions to permit fat hydrolysis and the fat recovered by churning or extraction.[12] The clear fat is mixed with a solvent and titrated with an alcoholic solution of alkali. Fat obtained by churning contains primarily the water-insoluble fatty acids, the water-soluble acids being lost, to some extent, in the buttermilk.

Surface Tension. The decrease in surface tension of a substrate, due presumably to liberation of free fatty acids, has suggested the use of surface tension measurements for determining lipolysis in certain dairy products. The method has various advantages[13] and limitations.[14]

Other Methods. Several other methods have been used to measure lipase activity. In some cases simple fatty acid esters, simple triglycerides, etc., have been used as a substrate. However, an enzyme capable of hydrolyzing some of the simpler compounds may not hydrolyze milk fat, and in dairy products hydrolysis of the fat is the important consideration.

Factors Increasing Lipase Activity. Lipolysis in milk may be accelerated in various ways.

Temperature Activation. Lipase activity is increased in certain samples of milk simply by cooling, and such activation is detected most readily by examining milk from individual animals. Herrington and Krukovsky[2] noted that there was little variation in the fat acidities of fresh milk from individual cows but considerable variation when tested after holding in ice water for 16 hr.

Lipase activity may be accelerated in raw milk by cooling, warming slightly, and recooling.[15] Maximum acceleration is obtained by cooling to 41 F or lower, warming to 86 F, and recooling to below 50 F. The period milk is held at the initial cooling or warming temperature is less important than the actual temperature employed. With this method of activation, some samples of milk become rancid within 1 hr, and most samples will be rancid within 24 hr.[8] Temperature activation may occur on farms when warm milk is mixed with cold milk in the proper proportion or in a plant when warming cold raw milk for

separation and later cooling the raw cream. Temperature activation occurs with products in which the fat globules still have their natural layer of adsorbed material.[16] Lipolysis in temperature-activated milk proceeds more rapidly at lower than at higher temperatures.

Homogenization. Size of the fat globules is an important factor in lipase activity. With small globules the surface area is relatively large and lipase activity is facilitated. Milk that is homogenized but not pasteurized quickly becomes rancid, and homogenized milk is sold only in a pasteurized condition. Trout[17] indicated that raw milk homogenized at 40, 60, or 140 F, using 5,000 lb pressure, does not develop rancidity in 24 hr, whereas milk homogenized at 80, 100, or 120 F develops rancidity in <2 hr. Gould[18] noted that raw milk showed the greatest lipolytic activity when homogenized at 105 to 125 F. Dorner and Widmer[19] found that when milk from individual animals was homogenized and held at 68 F, the time required for rancidity to develop varied widely.

Shaking or Churning. The action of lipase can be increased by shaking or churning raw milk or cream. Hlynka *et al.*[20] found that considerable fat dispersion takes place in vigorously agitated warm milk. Milk churned for 15 min at 86 F showed fat dispersion roughly equivalent to that of a mixture of 20% homogenized and 80% unhomogenized milk. Krukovsky and Sharp[21] stated that whether an increase in degree of dispersion is necessary to activate the lipase of milk by shaking or whether the activating mechanism is an alteration in the surface characteristics of the fat globules is not evident.

The effect of agitation by a pipe line milker is cited by Herrington.[14] A sample of an entire night milking was taken on 3 successive days at a farm where there were 5 risers in the pipe line. On the first and third days the milk traveled through the risers and pipe line to the milk house, and on the second day milkers were used but the milk was carried to the milk house. Fat acidities, after holding the milk for 36 hr, were 2.87, 0.92, and 2.59 on the first, second, and third days, respectively. It was difficult to understand why agitating warm raw milk with air in a vertical pipe should activate lipase much more than agitation for the same period in a Waring blendor; influence of air may have been a factor.

With homogenized, shaken, or churned milk or cream, lipolysis proceeds more slowly at lower than at higher temperatures.[8] In a comparison of 37.4 to 39.2, 80.6, and 98.6 F, lipolysis in sugar-saturated cream inoculated with milk was most extensive at 98.6 F, slightly less at 80.6 F, and decidedly less at 37.4 to 39.2 F.[3]

In many samples of milk from individual cows, and in practically all samples of mixed milk, lipase is quite inactive. However, certain methods of handling milk on the farm and in the dairy plant activate the enzyme or make conditions more favorable for fat hydrolysis.

Hydrogen-ion Concentration. The *p*H of the substrate has an influence on the rate of hydrolysis by milk lipase. Roahen and Sommer [3] adjusted cream to *p*H values of 6.19 to 8.91 and found that lipase activity increased with an increase in *p*H from 6.19 to 8.50. Maximum lipolysis occurred in the range 8.4 to 8.6.

Factors Tending to Decrease Lipase Activity. The fact that certain samples of milk from individual animals become rancid when held separately and do not cause rancidity when mixed with the milk of several animals suggests that lipase activity can be controlled to some extent by dilution. Tarassuk and Henderson [22] noted that diluting milk which would undergo spontaneous rancidity with four or five times its volume of normal milk prevented the defect.

Investigations indicate [23, 24] that spontaneous development of rancidity in milk can be prevented by holding fresh milk warm for several hours before cooling it. Peterson *et al.*[25] noted that some samples of milk lost most of the enzyme which hydrolyzed tributyrin when held for 10 hr at 35 F although other samples showed little or no loss.

Davies [26] found that traces of heavy metals in amounts up to 25 ppm inhibit lipase action in butter from unpasteurized sweet cream; degree of inhibition varied with the metal and its concentration. Copper was the most inhibitory of the metals studied; iron, nickel, cobalt, manganese, and chromium were less active; tin and aluminum had no effect. According to Krukovsky and Sharp,[27] normal milk lipase is susceptible to inactivation by dissolved oxygen, and this inactivation is accelerated by heat and by dissolved copper. In the absence of dissolved oxygen, dissolved copper caused no lipase inactivation in normal milk. Hlynka and Hood [28] reported that cysteine can reverse the inactivation of milk lipase brought about by aeration or aeration and copper. Anaerobic environment may bring about some reversibility of oxidatively inactivated milk lipase, but not in the presence of copper. Hlynka *et al.*[29] noted that, although copper inhibits lipase activity in milk under ordinary conditions, it is ineffective as a lipase inhibitor in cheddar cheese; this was attributed to the reducing potential of the cheese. Gould [30] found that copper had no significant effect on the extent of lipolysis when added prior to, or subsequent to, homogenization. Fat acidities of homogenized raw milk containing 2, 6, and 10 ppm copper were practically the same after 24 and 72 hr

as the control samples. The results indicated a difference in the action of copper on a homogenized and an unhomogenized product.

Herrington and Krukovsky [2] noted that 1 drop of formalin in 160 ml of 20% cream may reduce lipase action. However, even 27 drops did not prevent lipolysis entirely and were no more effective than a single drop. Gould [30] did not obtain decreased lipase action by addition of as much as 5 ml formalin to 3 lb cream obtained from homogenized raw milk. In 2 of 3 trials, a greater degree of fat hydrolysis occurred in the cream containing formaldehyde. Investigations indicate [3,25] that the enzyme capable of splitting tributyrin at *p*H 8.8 is sensitive to formaldehyde.

Sodium chloride is reported to decrease lipolysis in both homogenized and unhomogenized products.[5] Gould [30] found that addition of 2% salt to homogenized raw cream appreciably decreased lipolysis and 5 or 8% inhibited it almost completely.

Inactivation by Heat. In the investigations of Rogers,[31] action of milk lipase was weakened materially by an exposure of 10 min at 113 F and was destroyed entirely at 140 F for this period. Doan [32] heated milk of different fat contents under various conditions and homogenized it immediately at 2,000 lb pressure. Flash heating at about 147 F, 15 min at about 134 F, or 30 min at about 132 F prevented rancidity in the milk when it was held at 35 to 40 F for 24 hr. Trials by Tarassuk [33] indicated that lipase was partially inhibited by heating milk at 110 F for 30 min and completely inhibited at 130 F for 30 min. Hetrick and Tracy [34] homogenized raw milk and heated it within 5 sec after homogenization to various temperatures; the heating time was 0.83 sec. The temperatures and times required to prevent development of a rancid flavor were: 145 F, 8.03 min; 150 F, 2.0 min; 155 F, 22 sec; 160 F, 10 sec; 167 F, 2 sec; 173 F, 1 sec; and 186 F, 0.03 sec. At 145 F lipase was inactivated in about one-third the period required to inactivate phosphatase but at 185 F the same period was necessary to inactivate the 2 enzymes.

Nair [35] reported that lipase could not be detected in whole or skim milk powder desiccated by a drying system in which the fluid milk had undergone preliminary pasteurization at 145.4 to 148.1 F for 30 min. Krukovsky and Herrington [36] churned unsalted butter from fresh cream held at various temperatures for 0 to 150 min and measured the rate of lipolysis by titration of free acids in the fat. At 110 F lipolysis first was activated and then reduced as the holding increased; the rate was reduced about two-thirds by holding 150 min. At 125 F the rate of lipolysis was reduced about one-half after 20 min (esti-

mated) but still was measurable after 150 min. At 140 F the rate of lipolysis was reduced more than one-half at 0 holding time; it was measurable with 15 min holding but not with 35 min. At 155 F the rate of lipolysis scarcely was measurable after 0 min holding.

Relationship of the Animal to Rancid Milk. The amount of lipase in milk from individual animals varies and, in addition, its activity is influenced by various factors so that certain samples of raw milk slowly or rapidly become rancid, whereas others do not. Milk from cows advanced in lactation sometimes becomes rancid so quickly that it is practically unusable in a raw condition. Rancidity also occurs with milk from certain animals in middle or early lactation.[37,38] Mattick and Kay [39] found that colostrum milk was rich in lipase. Of 327 individual milk samples, representing all stages of lactation, the 7 most rancid samples came from cows which were in lactation for <10 days.

Kelly [40] noted a relationship between the estrus cycle of the cow and lipase activity of the milk. Freeden *et al.*[41] did not, but injections of pituitrin and stilbestrol caused an increase in lipolysis of milk with certain animals. Other studies [42] showed a maximum lipase content on the day of estrus, and, when estrus was induced by injections of diethyl stilbestrol, the milk became very rancid.

A marked decrease in lipase activity of milk was observed by Pfeffer *et al.*[5] when cows were taken from pasture and placed on dry feed. Such a decrease was not permanent, and milk returned to normal in about 2 weeks. A similar decrease was observed when cows were changed from dry feed to pasture. Tarassuk [33] worked with a Holstein cow that produced milk in which lipase was naturally active and found it possible to obtain milk high in lipolytic activity, or milk free from it, by changing from dry to green feed. The level of carotene [43] in blood or milk fat was not in itself the cause of a cow secreting milk which spontaneously developed rancidity.

Lipase in Mammary Tissue. Kelly [44] found that tissue from bovine mammary glands not developed by pregnancy contained only traces of active lipase, whereas tissue from developed glands contained significant amounts.

Lipase in Cream. Roahen and Sommer [3] reported that gravity-separated cream showed appreciably greater lipolytic activity than separator cream of the same fat content; loss of lipase in the separator slime, presumably, was a factor. Cream separated at 110 F showed less lipolysis than cream separated at 75 F; similarly, the 110 F skim milk caused less lipolysis than the 75 F skim milk.

Krukovsky and Sharp [16] noted that total lipolytic action increases with fat content up to 35 to 45%, but acidity per unit of fat or acidity per unit of plasma increases with fat content up to 8 to 10% and then remains constant, or decreases.

Cream in which lipase has been active commonly is difficult to churn. Palmer [45] suggested that this is due primarily to excessive foaming which accompanies formation of soaps from the fatty acids liberated. Krukovsky and Sharp [46] noted that the difficulty of churning and the foam formation in cream from cows in advanced lactation probably are due largely to the lipolytic action and concentration of the resultant soaps and fatty acids in the air-plasma interface; pasteurization of the milk or cream as soon as it was drawn largely prevented the difficulty in churning. Fouts and Weaver [37] found that cream from rancid milk samples required 10 to 500% longer to churn than cream from normal samples.

Lipases of Micro-organisms. Various micro-organisms hydrolyze the fat of dairy products in which they grow and may cause rancidity in milk, cream, butter, etc. (see p. 101).

PHOSPHATASE

Phosphatase hydrolyzes phosphoric acid esters with the liberation of inorganic phosphate and the alcohol. It regularly is present in raw milk and has been demonstrated in various ways, especially by determining the phenol liberated from disodium phenyl phosphate.[47]

Phosphatase assumed considerable importance when it was shown that its thermal stability is only slightly greater than that of *M. tuberculosis*. Over all temperature and time ranges, involving both LTLT and HTST pasteurization, *M. tuberculosis* is destroyed more quickly than phosphatase, so that a pasteurization exposure sufficient to inactivate phosphatase, to the degree called for by the standards which have been established for each method, assures destruction of the organism. Extensive investigations have resulted in great improvements in the methods of determining phosphatase activity in milk. Procedures have been developed that distinguish accurately between properly pasteurized and inadequately heated milk; they detect slight irregularities in the temperature or period of heating and also contamination with a very small percentage of raw milk, at least as little as 0.1%. Various phosphatase tests have been standardized [47] and they now constitute the accepted basis for determining whether pasteurization

has been carried out satisfactorily. Some of the tests can be used in field studies.

Phosphatase in Milk and Milk Products. The amount of phosphatase in raw milk varies with different animals and is influenced by the stage of lactation and other factors, for example, udder abnormalities. Wilson and Hart [48] found that the phosphatase activity of milk usually is higher than that of the blood plasma of the producing cow and also that activity in the milk is much greater in cows at the end of lactation than in heavy-producing cows at the beginning of lactation. In general, mixed herd milk varies little in phosphatase content.

Phosphatase is not fat soluble but apparently is associated with the protein layer of the fat globules. Hetrick and Tracy [49] studied the partition of phosphatase among various milk derivatives, the amounts being indicated by the phenol liberated from the substrates. Whole milk (3.6% fat) liberated 2,080 μg phenol per g and was separated into cream (41.5% fat) and skim milk (0.05% fat) which liberated 10,000 and 1,280 μg per g, respectively. Churning of the cream yielded buttermilk and butter (85% fat) which liberated 12,800 and 4,000 μg per g, respectively. Whey obtained from the skim milk by acid precipitation of the casein liberated 575 μg per g. Butteroil derived from the butter had no phosphatase activity whereas the butteroil sludge liberated 15,000 μg per g. When heated milk which liberated 7.5 μg phenol per g was separated, the resulting cream and skim milk liberated 12.8 and 5.4 μg per g, respectively. Properly pasteurized milk which liberated 3.3 μg phenol per g yielded cream and skim milk which liberated 4.9 and 2.2 μg per g, respectively.

Inactivation by Heat. Kay and Graham [50] noted that phosphatase is inactivated progressively as milk is heated. Later,[51] they presented 2 methods of determining the efficiency of pasteurization that are based on the effect of heat on phosphatase.

Sanders and Sager [52] noted that the exposures required to reduce phosphatase activity to 4 μg phenol per g in whole milk were: 37.5 min at 143 F, 30 min at 143.7 F, 24 sec at 160 F, and 15 sec at 161.8 F. The temperature required to reduce the activity to 4 μg per g in any given time was about 0.7 F lower for skim milk than for whole milk, about 4.5 F higher for ice cream mix than for whole milk, and about 5.7 F higher for sherbet than for whole milk. The time required at 143 F was about 3 times as long for ice cream mix as for whole milk. A milk sample, adjusted to different *p*H levels and heated, showed that milk phosphatase was more stable towards heat when the *p*H was within the range of 6.5 to 7.4.

According to Hetrick and Tracy,[49] the following exposures reduce the phosphatase activity of milk to 1 μg per g when the time to heat is 0.8 sec: 7 min at 150 F, 1.86 min at 155 F, >22 but <32 sec at 160 F, 10 sec at 165 F, 1 sec at 174 F, and 0.3 sec at 185 F. Hansen *et al.*[53] studied inactivation of phosphatase using a HTST pasteurizer of 1,000 lb per hr capacity and a rated holding time of 15 sec. With this equipment phosphatase activity was reduced to 4 μg per g at an average temperature of 157.7 F, the inactivation time being 29.2 sec (time milk was above control temperature plus heating and cooling equivalents). When the control temperature was 160 F, the holding and inactivation times were 9.6 and 16.8 sec, respectively. With the milk flow increased by 60%, phosphatase was inactivated at 159.7 F with a holding time of 10.85 sec. The rate of inactivation of phosphatase at any temperature was logarithmic until a low residual phosphatase level was reached.

Fay [54] stated that more heat is required with cream than with milk to meet the phosphatase standard for pasteurized milk. Gilcreas [55] obtained positive phosphatase tests on cream separated from milk pasteurized at 143 F for 30 min; the pasteurized milk and skim milk gave negative tests. Trembath and Newman [56] reported that cream heated to 145.4 F for 30 min invariably exceeded the phosphatase standard for milk although other investigators [57] found negative results with this exposure. According to Crossley and Rothwell,[57] the minimum exposure for cream with HTST pasteurization, to meet the standard for milk, is 165.2 F for 15 sec.

Phosphatase appears to be inactivated at considerably lower temperatures and shorter holding times in cheddar cheese than in milk.[52] Inactivation of the enzyme in cheese at *p*H 5.29 occurred in 13 min at 130 F and in <0.75 min at 140 F. Addition of alkali to cheese to increase the *p*H tended to stabilize the enzyme against heat. Adding emulsifiers had an even greater effect as about 0.5 min at 150 F was required to give a negative test when 1.5% anhydrous disodium phosphate was added and the *p*H of the mixture was 5.56. Addition of lactose to cheese increased the stability of the enzyme whereas water decreased it.

Reactivation of Phosphatase. Samples of milk and cream heated to high temperatures for very short periods, and which gave negative phosphatase tests immediately after heat treatment, have given positive tests after holding. Brown and Elliker [58] noted that reactivation occurred in cream held at 40 F, but it was more pronounced when held at higher temperatures. Posthumus [59] obtained positive tests

after holding cream at 68 F for >3 hr. According to Ritter,[60] no reactivation occurs in cream heated by LTLT pasteurization, or by slow heating to 161.6 or 167 F, but it does occur with cream subjected to HTST pasteurization. Other investigators [57] did not observe reactivation of phosphatase in LTLT pasteurized cream or in cream held at 165.2 F for 15 sec. However, a large percentage of samples heated to 179.6 F, or higher, showed reactivation after holding at 68 F for 48 hr.

Wright and Tramer [61] noted that samples of milk sterilized by rapid heating to high temperatures developed a positive phosphatase reaction on storage. The optimum storage temperature for reactivation was 86 F. The samples remained sterile during storage. Phosphatase reactivation occurred at all temperatures of heating from 185 to 356 F but depended on the holding time at these temperatures. No evidence of reactivation occurred in either commercial samples of pasteurized milk (161 F for 15 sec or 145 F for 30 min) or laboratory pasteurized milk (145 F for 30 min).

Preservatives for Milk to be Tested for Phosphatase. A number of compounds were tested by Sanders and Sager [62] for use as preservatives for dairy products to be tested for phosphatase. The compounds, arranged in increasing order of their inhibitory effect on milk phosphatase, were: 1.5 to 2.0% chloroform, 3.0 to 3.5% toluene, 1.5 to 2.0% sodium tetraborate, 0.01 to 0.03% formaldehyde, 0.05 to 0.1% mercuric chloride, and 0.06 to 0.15% hydrogen peroxide. Chloroform was more effective than toluene as a preservative in the same concentration, but neither compound inhibited the enzyme appreciably. Chloroform also prevented the fat emulsion from breaking and thus eliminated lumps of fat from the surface of milk. Hydrogen peroxide, mercuric chloride, and formaldehyde seriously inhibited milk phosphatase. For fluid dairy products the authors recommended preservation with 1.5 to 2.0% chloroform. For solid dairy products, such as cheese, they recommended that chloroform be added in quantity equal to 1.5 to 2.0% of the volume of the container; adding the chloroform to cotton placed in the container was suggested.

Phosphatase Production by Micro-organisms. The production of phosphatase in milk by various organisms is of special importance. Leahy *et al.*[63] found, with 28 cultures belonging to the genera *Lactobacillus, Streptococcus, Micrococcus, Escherichia, Aerobacter, Salmonella, Klebsiella,* and *Pseudomonas,* that 1 strain of *Micrococcus,* 5 strains of *Aerobacter,* and 3 strains of *Klebsiella* produced a true phosphatase reaction; additional strains produced a false reaction. The

authors suggested that phosphatase tests on milk having microscopic counts of >8 million per ml or plate counts of >2 million should be interpreted with caution.

Olson and Hammer [64] noted that various organisms produced phosphatase in sterile milk. Some of the species of the genus *Pseudomonas* were most active in this respect. An important point in connection with the formation of phosphatase in dairy products by bacteria is the ability of various *Pseudomonas* organisms to grow at relatively low temperatures.

Buck [65] reported a positive phosphatase test on properly pasteurized, commercial, bottled milk. The bacterial phosphatase responsible was produced by a thermophile which was named *Lactobacillus enzymothermophilus.* Factors governing production of the phosphatase were size of inoculum, temperature, and holding period. The bacterial enzyme was destroyed at 185 F for 1 min. Barber and Frazier [66] reported that lots of commercially pasteurized cream which originally were phosphatase negative became positive during 3 to 4 days at 39.2 to 50 F and while still in a salable condition. The phosphatase-positive samples contained organisms that produced phosphatase; they were identified as *B. cereus* and *Bacillus mesentericus.* When pure cultures of the organisms were inoculated into cream, the rapidity with which a positive phosphatase reaction developed depended on the numbers of organisms and amounts of bacterial phosphatase added by the inoculum. A positive reaction was obtained on pasteurized cream when the microscopic count of the organisms was between 1.48 and 7.8 million per ml. Bacterial phosphatase and milk phosphatase were active in the same general *p*H range, but in cream bacterial phosphatase withstood exposures as high as 170 F for 30 min while milk phosphatase was inactivated at 145 F for 30 min.

PROTEASE

Babcock and Russell [67] reported that in milk to which chloroform or ether had been added coagulation and an increase in soluble nitrogen eventually occurred. Their studies indicated that precipitation of the casein was influenced by the temperature of holding more than anything else. They made enzyme preparations that had a curdling as well as a digesting action on milk. Babcock *et al.*[68] proposed the name galactase for the coagulating and digesting enzyme and presented data suggesting that the curdling action is independent of the

peptonization. Their results further show that activity of the enzyme is retarded materially in the presence of acid. Protease is now the preferred designation for the enzyme. Babcock *et al.*[69] studied protease in comparison with various enzymes and cultures of bacteria from the standpoint of decomposition products in milk. They concluded that the products formed by it approach more nearly those formed by the peptonizing bacteria than by any of the enzymes of animal origin.

Protease can be demonstrated in milk by treating a sample with chloroform or ether, stoppering tightly to prevent loss of the reagent, and then holding at a favorable temperature, such as 98.6 F. The milk usually forms a soft curd in 1 to 3 weeks; the curd gradually acquires a somewhat transparent appearance, presumably due to proteolysis. Soluble nitrogen determinations on the fresh and curdled milk show the extent of the decomposition. Protease also can be demonstrated at 70 F but it is less active there than at 98.6 F.

The presence of protease has been noted in milk of various species,[70] including man, sheep, goat, pig, horse, burro, and a half-breed buffalo. During the separation of cows' milk, protease [71] is highly concentrated in the slime and slightly concentrated in the cream. The enzyme is active [68] in the presence of chloroform, ether, benzene, or toluene, but mercuric chloride, formaldehyde, phenol, or carbon bisulfide destroy it; 1% chloroform [71] in skim milk or buttermilk was found to inhibit proteolysis entirely, whereas smaller amounts permitted it. Proteolysis is more active in slightly alkaline or neutral reaction but also occurs when some acid is present. Protein decomposition brought about by protease goes to the amino acid stage.

Babcock *et al.*[68] found that heating for 10 min at 168.8 F destroyed protease and that 10 min at 159.8 F materially reduced its activity. Rogers *et al.*[72] reported that with flash pasteurization protease was much weakened between 159.8 and 170.6 F but was not destroyed at 199.4 F, the highest temperature employed. Protease evidently is the most heat resistant of the milk enzymes.

Protease appears to be an important factor in the ripening of various hard cheeses, its role being to aid in the breakdown of the complex nitrogenous constituents. This decomposition is necessary for the proper development of texture and flavor. Because of the curd in butter, action of protease also must be considered as a possible factor in deterioration of this product. Thatcher and Dahlberg [71] obtained a uniform and measurable proteolytic action from two samples of

fresh dairy butter, but none from fresh creamery butter. The investigators noted that 15% sodium chloride in skim milk entirely prevented the activity of protease, which led them to conclude that it cannot act in normal salted butter because of the salt content.

Warner and Polis [73] found that in solutions of casein an enzyme caused slow hydrolysis, a decrease in viscosity, and appearance of nitrogenous products soluble at *p*H 4.6 and in trichloroacetic acid. Presumably, the enzyme is the same as that studied by Babcock *et al.* It was present in all samples of commercial casein and in all laboratory preparations made by the usual methods; since it was present with either acid or rennet precipitation, it would be present in cheeses. Optimum activity was at *p*H 8.5; it was active at *p*H 5.8, which was the lowest *p*H at which satisfactory solutions could be prepared, and at *p*H 11.2.

Proteases of Micro-organisms. The proteolytic enzymes produced by organisms which decompose casein are important with various dairy products. Many organisms which produce lipase also produce protease.

Chopra [74] noted that the proteolytic activity of cell-free filtrates from *Bacillus thermophilus, Bacillus aerothermophilus,* and *B. coagulans* cultures were most active against gelatine at *p*H 8.0 and against casein at *p*H 7.5. When the organisms were grown in the presence of carbohydrate, proteolytic activity of the filtrates was decreased, but formation of intracellular proteases was not inhibited.[75] Production of protease by *B. subtilis* is favored by the presence of manganese ions during growth.[76] Gorini [77] studied the proteases of several *Micrococcus* and *Bacillus* species. All of them were most active between *p*H 7 and 8. Reagents which formed complexes with calcium, such as fluoride, citrate, oxalate, nitrilotriacetate, ethylenediamine tetraacetate, and hexametaphosphate, inhibited protease activity; hexametaphosphate was the most effective. Inactivation by these compounds could be reversed by the addition of calcium when the compounds were in contact with the enzyme for a very short time, but prolonged contact with the inhibitors was not reversible by calcium. Magnesium, and to some extent manganese, could protect the enzyme against inhibition but could not restore the activity of the enzyme as calcium did. Calcium also protected the enzyme against heat inactivation. The author concluded that calcium is a normal constituent of bacterial proteases and is required for stability and activity.

PEROXIDASE

Peroxidase oxidizes readily oxidizable substances by transferring oxygen to them from some peroxide, for example hydrogen peroxide. It can be demonstrated in milk by adding to a small volume a few drops of hydrogen peroxide and some indicator which readily is oxidized and which gives a pronounced color when changed. Satisfactory indicators are freshly prepared 10% gum guaiac in alcohol, 1.5% aqueous solution of ortol, 2% aqueous solution of paraphenylenediamine, etc.; a large number of indicators have been employed, some of which are mixtures of reagents. The indicator often is added before the hydrogen peroxide so that oxidases can be detected. Van der Smissen [78] found peroxidase activity lower in raw whole milk than in skim milk separated from it; cream added to skim milk reduced its peroxidase activity. Schwarz and Sydow [79] noted that the peroxidase value of rennet whey from pasteurized milk was higher than that of the milk.

Elliott [80] obtained a crude but concentrated preparation of milk peroxidase, practically free of catalase, by fractional precipitation with ammonium sulfate. The activity decreased very slowly at 32 to 41 F. The enzyme was active from about *p*H 4 to nearly 10. From *p*H 8 to nearly 10 activity was diminished but recovered immediately on neutralizing. The enzyme oxidized nitrite quantitatively, as shown by usage of hydrogen peroxide or disappearance of nitrite. Using guaiacol as the substrate, Rozental [81] found the optima for milk peroxidase to be *p*H 8.30 and 78 F.

Buruiana [82] proposed a peroxidase index (100 $\times$ log of the reciprocal of the maximum dilution which just gives an enzyme reaction) for measuring peroxidase activity. The indexes on 32 samples of cow milk varied from 210 to 231, on human milk from 30 to 361, on buffalo milk from 240 to 602, on sheep milk from 180 to 361, and on ass milk from 60 to 180.

Destruction by Heat. Rogers *et al.*[72] found that in cream pasteurized with the flash method peroxidase was absent after heating to 174.2 F, or above; at 170.6 F it generally was absent; at 165.2 F, or below, it always was present. Spitzer and Taylor [83] reported that 144.5 F for 20 min had no apparent effect on the peroxidase in milk, whereas flash pasteurization at 185 F inactivated the greater part of it.

Importance of Peroxidase. The peroxidase of milk affords a basis for determining whether milk and its various derivatives have been

heated to a comparatively high temperature. This is important in enforcing certain pasteurization regulations, especially those dealing with pasteurization of factory by-products, but cannot be used to show whether market milk has been heated to the exposures usually employed because of the temperature required to destroy peroxidase. It is probable that in certain instances pasteurization requirements intended to prevent spread of animal diseases through skim milk, etc., were selected with the idea of the presence of peroxidase providing a means of detecting violations.

At one time a peroxidase test (Storch test) was used on butter in Canada where it was considered that, when cream has been pasteurized at 170 F for 10 min, or longer, or at a higher temperature, peroxidase was destroyed. In making the test [84] about 2 oz of butter in a sample jar is melted in a water bath at 120 to 130 F. When the serum has separated about 4 to 5 ml is pipetted into a test tube. This is diluted with an equal volume of warm water. Two drops of hydrogen peroxide (1 vol of 3% peroxide and 2 vol distilled water) are added and then immediately 2 drops of paraphenylenediamine solution (2 g dissolved in 100 ml distilled water at 140 F). A decidedly blue color within 0.5 min indicates the sample contains peroxidase and the cream was not pasteurized; no color indicates the sample contains no peroxidase and the cream was pasteurized.

Peroxidase in butter sometimes seems to disappear on holding. Rogers *et al.*[72] found that in buttermilk it disappeared in about half the samples during holding for 1 yr and ascribed this to the slow digestion taking place. They stated that the peroxidase reaction in raw milk can be made to disappear in 20 or 25 days by permitting the milk to sour, adding chloroform to eliminate subsequent bacterial action, and then allowing proteolysis by the lactic acid to go on, with or without aid of pepsin; they also pointed out that salts of iron may be responsible for a reaction indistinguishable from a peroxidase reaction.

Dahle and Palmer [85] investigated the effect of storage on peroxidase activity of whole-milk powders and concluded that factors known to favor oxidation, such as air, heat, and moisture, proved detrimental to it. Powders stored in vacuum or in containers which prevented entrance of air and moisture showed greater peroxidase activity than the same powders exposed to air and moisture.

In view of the limited oxidizing power of milk peroxidase, it would be expected to have little influence on the deterioration of dairy products. Palmer and Miller [86] found that addition of peroxidase from

horse-radish roots to pasteurized cream had no effect on keeping quality of the butter churned from it, either with or without ripening of the cream; the butter was stored at room temperature and at 32 F.

Peroxidase may be of significance from the bacteriological standpoint. Demeter and Steude [87] reported that a substance hastening the killing of milk bacteria by heat was removed from milk by means of kaolin and 5% ammonium chloride solution and found to be peroxidase. The substance gave the guaiacol reaction and was destroyed at 176 F. Added to peroxidase-free milk, it shortened the period required to kill bacteria at 143.6 F. Since bacteria of the coliform group destroy peroxidase, the accelerating effect may be absent from milk containing large numbers of these organisms.

Peroxidase Production by Micro-organisms. Peroxidase is produced by many bacteria. In general, many aerobic bacteria exhibit peroxidase activity while the anaerobes are inactive.

CATALASE

Catalase splits hydrogen peroxide into water and molecular oxygen according to the reaction $2H_2O_2=2H_2O + O_2$. Its presence in raw milk can be shown by adding a small amount of hydrogen peroxide and observing the liberation of bubbles of oxygen. Use of a definite volume of milk with a standard amount of hydrogen peroxide and a tube in which the liberated gas can be collected and measured permits a comparison of the activity of the catalase in various lots of milk.

Catalase is distributed widely in nature and is present in the milk of a variety of animals. Catalase contents of various lots of milk differ considerably, with colostrum and milk from cows advanced in lactation containing especially large amounts. Milk from abnormal udders often shows excessive amounts of catalase because of the presence of many leucocytes. Cream and separator slime contain more catalase than milk. Thatcher and Dahlberg [71] examined a number of samples of butter and noted that each contained catalase; storage did not appear to decrease its activity. Rogers *et al.*[72] found catalase was destroyed in cream pasteurized by the flash method at 158 F.

Catalase apparently is of little importance in milk or its derivatives. Since excessive amounts sometimes are due to large numbers of bacteria or leucocytes or to some other objectionable condition, determination of the catalase activity of milk has been suggested as a test for the sanitary quality. Such a determination also has been proposed

as a test for pasteurization since catalase is influenced at the temperatures commonly used in pasteurizing market milk.

In attempts to use hydrogen peroxide in milk for various purposes, the excess peroxide sometimes has been removed by adding commercial catalase.

Production of Catalase by Micro-organisms. Certain organisms produce catalase so that their action may be a factor when the test material contains many of them. Sherman [88] reported that swiss-type cheese contains more catalase than cheddar cheese because of its characteristic bacterial flora. Catalase is produced by practically all aerobic bacteria but not by anaerobic bacteria. Several facultative anaerobes produce the enzyme.

OXIDASE

The presence of oxidase in milk has been demonstrated in various ways. When an aldehyde is added to raw milk, methylene blue is reduced to its leuco base; the reaction does not occur with milk heated to 170 F for 15 min.[89] Oxidase also may be demonstrated in milk by its ability to oxidize hypoxanthine, or xanthine, to uric acid. Dixon and Thurlow [90] concluded both reactions are due to the same enzyme, whereas Wieland and Mitchell [91] presented data indicating they are not. Greenbank *et al.*[92] noted that the enzyme oxidizing xanthine is not specific but promotes oxidation of numerous aldehydes. Addition of inhibitors for the enzyme to pasteurized milk before drying gave a dried milk of superior keeping quality to that prepared without inhibitor.

Oxidase Production by Micro-organisms. Production of oxidase by micro-organisms is detected by color changes in the colonies when treated with such indicators as *p*-aminodimethylaniline hydrochloride and tetramethyl-*p*-phenylenediamine hydrochloride (p. 18). Bacteria vary widely in their oxidizing powers, and some of the strongly oxidizing types produce objectionable changes in certain dairy products. Castell and Garrard [93] found that *Pseudomonas* and *Achromobacter* organisms are the most strongly oxidizing types; *Alcaligenes* and *Brucella* organisms are somewhat less but still strongly positive; *Aerobacter, Escherichia,* and *Proteus* organisms are weakly positive, variable, or negative; *Bacillus* organisms are weakly positive or negative; cocci and the one anaerobe studied were definitely negative. All strong oxidizers are gram negative and definitely not strong oxidizers are gram positive. The authors noted that the organisms which

produce the more serious flavor defects in butter include almost wholly the strongly oxidase-positive bacteria.

In milk the oxidase-producing bacteria apparently do not produce oxidized flavor. Cone and Babcock [91] inoculated 9 oxidase-producing bacteria into freshly pasteurized milk which was susceptible to development of oxidized flavor, either spontaneously or with addition of no more than 0.1 ppm copper. Amount of inoculation varied from <100 to several million organisms per ml. None of the inoculated milk developed a greater intensity of oxidized flavor than the control when held 4 days at 39.2 to 42.8 F; with large inocula, development of oxidized flavor was markedly or completely inhibited. There was no evidence that oxidase-producing bacteria differ greatly from other bacteria with respect to oxidized flavor in milk.

Storgårds and Hietaranta [95] noted that oxidase-positive bacteria, especially a *Micrococcus* and a *Pseudomonas*, showed intense peroxide formation in butter. Formation of peroxide was particularly pronounced when the organisms were worked into butter.

ADDITIONAL ENZYMES IN MILK

Milk contains several enzymes which appear to be of minor importance. Amylase is present in the lactoglobulin fraction of milk serum proteins.[96] Salolase hydrolyzes phenyl salicylate.[97] A lactase has been reported in milk.[97]

REFERENCES

1. *Wis. Agr. Expt. Sta., 14th Ann. Rept.*, 211 (1897)
2. *J. Dairy Sci.*, **22**, 127 (1939).
3. *J. Dairy Sci.*, **23**, 831 (1940).
4. *J. Dairy Sci.*, **37**, 644 (1954).
5. *J. Dairy Sci.*, **21**, A143 (1938).
6. *Milk Plant Monthly*, 38 (10), 48 (1949).
7. *J. Dairy Sci.*, **5**, 64 (1922).
8. *Conv. Proc. Milk Ind. Foundation*, **4**, 30 (1950).
9. *J. Dairy Sci.*, **27**, 173 (1944).
10. *J. Agr. Research*, **52**, 49 (1936).
11. *J. Dairy Sci.*, **21**, 335 (1938).
12. *J. Dairy Sci.*, **32**, 435 (1949).
13. *Pa. Agr. Expt. Sta. Tech. Bull.* 287 (1933).
14. *J. Dairy Sci.*, **37**, 775 (1954).
15. *J. Dairy Sci.*, **22**, 137 (1939).
16. *J. Dairy Sci.*, **23**, 1109 (1940).
17. Trout, G. M. *Homogenized Milk*, 60, 1950. Michigan State College Press, East Lansing, Mich.
18. *Ind. Eng. Chem.*, **32**, 876 (1940).
19. *Lait*, **11**, 545 (1931).
20. *J. Dairy Sci.*, **28**, 79 (1945).
21. *J. Dairy Sci.*, **21**, 671 (1938).
22. *J. Dairy Sci.*, **25**, 801 (1942).
23. *Science*, **93**, 310 (1941).
24. *Can. Dairy Ice Cream J.*, **25** (6), 27 (1946).
25. *J. Dairy Sci.*, **26**, 233 (1943).
26. *J. Dairy Research*, **3**, 254 (1932).
27. *J. Dairy Sci.*, **23**, 1119 (1940).

28. *J. Dairy Sci.*, **25**, 111 (1942).
29. *J. Dairy Sci.*, **25**, 923 (1942).
30. *J. Dairy Sci.*, **24**, 779 (1941).
31. *U. S. Dept. Agr. Bur. Dairy Ind. Bull.* 57 (1904).
32. *Milk Dealer*, **23** (11), 40 (1933).
33. *Bull. Intern. Assoc. Milk Dealers 32nd yr.*, 153 (1939).
34. *J. Dairy Sci.*, **31**, 881 (1948).
35. *Ind. Eng. Chem.*, **22**, 42 (1930).
36. *J. Dairy Sci.*, **25**, 231 (1942).
37. *J. Dairy Sci.*, **19**, 482 (1936).
38. *J. Dairy Sci.*, **27**, 683 (1944).
39. *J. Dairy Research*, **9**, 58 (1938).
40. *J. Dairy Sci.*, **28**, 803 (1945).
41. *J. Dairy Sci.*, **34**, 521 (1951).
42. *Md. Agr. Expt. Sta., 65th Ann. Rept.*, 43 (1951–52).
43. *J. Dairy Sci.*, **26**, 987 (1943).
44. *J. Dairy Sci.*, **26**, 385 (1943).
45. *J. Dairy Sci.*, **5**, 201 (1922).
46. *J. Dairy Sci.*, **19**, 279 (1936).
47. *Standard Methods for the Examination of Dairy Products.* (Last ed.) American Public Health Association, Inc., New York, N. Y.
48. *J. Dairy Sci.*, **15**, 116 (1932).
49. *J. Dairy Sci.*, **31**, 867 (1948).
50. *J. Dairy Research*, **5**, 63 (1933).
51. *J. Dairy Research*, **6**, 191 (1935).
52. *J. Dairy Sci.*, **31**, 845 (1948).
53. *Can. J. Technol.*, **31**, 240 (1953).
54. *Proc. Short Course Conf. and Ann. Meeting Vt. Dairy Plant Operators*, **17** (1938).
55. *Am. J. Public Health*, **29**, 158 (1939).
56. *J. Dept. Agr., Victoria*, **36**, 88 (1938).
57. *Dairy Sci. Abstr.*, **16**, 779 (1954).
58. *J. Bacteriol.*, **43**, 118 (1942).
59. *Neth. Milk Dairy J.*, **6**, 361 (1952).
60. *Intern. Dairy Congr. Proc. 13th Congr.*, **3**, 1014 (1953).
61. *J. Dairy Research*, **20**, 177 (1953).
62. *J. Dairy Sci.*, **32**, 166 (1949).
63. *J. Milk Technol.*, **3**, 183 (1940).
64. *J. Milk Technol.*, **4**, 83 (1941).
65. *Am. J. Public Health*, **32**, 1224 (1942).
66. *J. Dairy Sci.*, **26**, 343 (1943).
67. *Wis. Agr. Expt. Sta., 14th Ann. Rept.*, 161 (1897).
68. *Wis. Agr. Expt. Sta., 15th Ann. Rept.*, 77 (1898).
69. *Wis. Agr. Expt. Sta., 16th Ann. Rept.*, 157 (1899).
70. *Wis. Agr. Expt. Sta., 15th Ann. Rept.*, 93 (1898).
71. *J. Agr. Research*, **11**, 437 (1917).
72. *U. S. Dept. Agr. Bur. Animal Ind. Circ.* 189 (1912).
73. *J. Am. Chem. Soc.*, **67**, 529 (1945).
74. *Proc. Indian Acad. Sci.*, **B23**, 100 (1946).
75. *Proc. Indian Acad. Sci.*, **B23**, 323 (1946).
76. *J. Bacteriol.*, **52**, 227 (1946).
77. *Biochim. et Biophys. Acta*, **6**, 237 (1950).
78. *Milchwirtsch. Forsch. Ber.*, **3**, 73 (1951).
79. *Milchwirtsch. Forsch. Ber.*, **6**, 367 (1954).
80. *Biochem. J.*, **26**, 10 (1932).
81. *Roczniki Panstwowego Zakladu Hig.*, **2**, 319 (1951).
82. *Lait*, **29**, 562 (1949).
83. *J. Dairy Sci.*, **7**, 234 (1924).
84. *Can. Dept. Agr. Bull.* 138, n.s. 43 (1930).
85. *J. Dairy Sci.*, **7**, 141 (1924).
86. *J. Dairy Sci.*, **9**, 272 (1926).
87. *Milchwirtsch. Forsch.*, **19**, 122 (1937).
88. *J. Dairy Sci.*, **2**, 453 (1919).
89. *J. Dairy Sci.*, **37**, 644 (1954).
90. *Biochem. J.*, **18**, 976 (1924).
91. *Ann.*, **492**, 156 (1932).
92. *J. Dairy Sci.*, **37**, 644 (1954).
93. *Food Research*, **5**, 215 (1940).
94. *J. Dairy Sci.*, **26**, 1 (1943).
95. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 280 (1949).
96. *J. Dairy Sci.*, **37**, 644 (1954).
97. *Biochem. Z.*, **11**, 61 (1908).

Bacteriology of Evaporated Milk

11

Large volumes of milk are converted to evaporated milk in order to supply areas in which fresh milk is not readily available and also to provide milk that can be stored on the same basis as many other foods. The keeping properties depend on destruction of the contained organisms during the sterilization and, when this actually occurs, there is no spoilage due to micro-organisms. However, bacteria occasionally survive the heat treatment and some of them can cause abnormal conditions in the product. The water and nutrients in evaporated milk make it well suited to growth of many types of organisms and, since nothing is added to control growth, changes often take place when the heat treatment is incomplete. In general, organisms which survive the heat treatment are spore-forming bacteria that are relatively unimportant in other dairy products so that evaporated milk presents a more or less characteristic bacteriological problem.

Occasionally, a can of evaporated milk spoils as a result of entrance of organisms through a defect in the tin. Under these conditions non-spore-forming bacteria commonly are involved.

Bacteriological Examination. In the bacteriological examination of a can of evaporated milk, great care is needed in opening in order to avoid contamination. The can should be agitated, washed thoroughly, and dried with a clean cloth. Then, a small area can be

flamed, covered with alcohol and the alcohol burned, or flooded with solder. The can is punctured in the treated area with a flamed piece of metal, such as a nail. Material is taken from the can with a sterile glass tube drawn to a fine bore so the puncture can be kept small. It can be inoculated into milk or any desired liquid medium, or smeared on agar, and incubated aerobically and/or anaerobically. Plate counts can be made but, ordinarily, are of no particular value. Often it is desirable to reseal the can for further tests, and this is done easily by soldering if an area on the can was flooded with solder.

Material from a can of spoiled evaporated milk can be stained and examined microscopically. Ordinarily, spoilage organisms are seen readily. With certain stains and careful observations, a portion of the organisms that were killed by the heat treatment can be seen; normally, these are not confused with active spoilage types because of their poor staining properties. Leucocytes in evaporated milk also are readily observed in stained preparations.

Normal Evaporated Milk. When cultured in milk or on agar, normal evaporated milk commonly does not yield organisms. Weinzirl [1] found no living organisms in 23 cans. Deming and Davis [2] studied 154 cans, using anaerobic and aerobic cultures at 98.6 F (37 C) with 102 of them and at 131 F (55 C) with 104. No growth was obtained except in one anaerobic culture at 131 F which showed a spore-forming rod; attempts to perpetuate the culture were unsuccessful. Nichols [3] found 35 cans of evaporated milk from general sources were sterile. In the laboratories maintained by certain evaporating plants, cultures prepared in checking sterilization efficiencies normally are negative.

Occasionally, evaporated milk that is normal in appearance readily yields bacteria on culture. Certain of them, although developing large colonies on agar, produce little if any change in milk, and it appears that milk is not a satisfactory medium for them; organisms with these general characters have been found for extended periods in the product of a plant without causing defects. Other organisms in evaporated milk apparently are prevented from growing, or are greatly limited in their growth, by lack of air; when air is provided growth is rapid and conspicuous changes occur. Still others have such high growth temperatures that they do not develop under the usual holding conditions for evaporated milk. In some instances normal-appearing evaporated milk has contained surprisingly large numbers of bacteria.

Theophilus and Hammer [4] found 1 of 15 cans (7 brands) of evaporated milk yielded colonies on culture, all of which appeared to be of one species; odor and flavor of the milk were normal. Spores of the

organism were destroyed by heat rather easily. Nichols [3] cultured evaporated milk and canned cream from selected plants. With 235 cans of milk and 52 cans of cream from one factory, 94 and 85%, respectively, were sterile; in another factory the percentages were 93 (with 44 cans) and 100 (with 6 cans); for still another factory they were 100 (with 1 can) and 76 (with 178 cans). It appeared the comparatively low sterility percentages for some samples were related to special circumstances of operation. In one factory more than one-half the unsterile cans were produced during a 10-day period when processing apparently was not carried out as efficiently as usual. Samples examined because they were obviously defective, or associated with defective packs, showed a much higher percentage of unsterile cans.

Sterilization Exposures. With evaporated milk, the proper sterilization exposure is very important. The heating must not be too severe because of the undesirable chemical changes that result, but it should destroy all the bacteria present. No general statement can be made as to the exposure required since it varies with a number of factors, and an exposure that is entirely satisfactory under one set of conditions may not be under another. Because of the development of some unusual situation, it sometimes is necessary to abandon an exposure that has been satisfactory over long periods; most often the exposure is increased in an attempt to destroy some resistant organism that suddenly has caused spoilage. After the heating, the cans of milk are cooled rapidly.

Each evaporated milk plant has developed a sterilization procedure, either batch or continuous, which appears to be satisfactory for its conditions. The exposures used commonly are varied slightly with the different sizes of cans and sometimes with the quality of the original milk. A plant may use various procedures that it considers important in carrying out the sterilization. In some instances these include use of relatively low heat treatments in order to limit heated flavor and darkening and thus obtain a product that is especially pleasing to consumers.

It appears that relatively high temperatures for relatively short periods offer some interesting possibilities in connection with the sterilization of evaporated milk, especially when a system which adds the heated and cooled milk to sterile cans (aseptic canning) is used.

Factors Influencing Heat Resistance of Spores. From the exposures used it is evident vegetative cells never survive the heat treatment of evaporated milk and only spores can cause difficulty in this way. Var-

ious factors undoubtedly influence the heat resistance of spores of a given species. Although some of the factors are of little importance in evaporated milk, because of its relatively constant composition and the uniform methods of production, others may be of significance.

In studying the influence of various factors on heat resistance of spores, Curran [5] noted that aqueous suspensions of spores subjected to long-continued freezing at 5 F became less heat resistant, whereas alternate freezing and thawing were not so effective in reducing resistance. Storage of spores at moderate temperatures was more conducive to enhanced resistance than storage at low or high temperatures. Prolonged action of metabolic products tended to reduce the resistance materially. Spores formed and aged in soil or in oats were much more heat resistant than those formed and aged in broth or on agar.

Theophilus and Hammer [4] found with certain aerobic, spore-forming bacteria isolated from normal and spoiled evaporated milk and from raw milk that growth at the approximate optimum temperature commonly yielded spores with greater thermal resistance than growth below or above that temperature. Sudden decreases in growth temperature from the approximate optimum usually decreased the resistance of the spores produced and sudden increases to the approximate optimum usually increased the resistance. Comparisons of the resistance of moist and freshly dried spores (6 to 8 days) indicated there was no regularity in the effect of drying; growth temperature influenced the resistance of dried spores in the same manner as moist spores. Aging of dried spores (47 to 69 days) did not significantly decrease the resistance. A strain of *Bacillus megatherium* isolated from spoiled evaporated milk showed a comparatively high thermal resistance, whereas a strain isolated from raw milk did not.

Curran and Evans [6] reported that sublethal heating of the spores of thermotolerant and thermophilic aerobes has an influence on the number of spores which germinate subsequently. They found with 12 thermotolerant cultures isolated from 10 outbreaks of spoilage in evaporated milk that nearly all would respond to pregermination heating; in the absence of such treatment a large proportion of the potentially viable spores did not germinate. The proportion of spores which responded to preheating was dependent on amount of heat, composition of the medium in which they were heated, temperature at which the spores were formed, and temperature at which they were subcultured. The authors suggested that, in the manufacture of evaporated milk, forewarming the milk at 203 F for 10 min prior to condens-

ing results in the majority of the spores of thermotolerant species becoming heat labile during the period the milk is being condensed and that, as a consequence, they are killed easily by the subsequent processing; thus an established commercial practice conduces to a desired result. Later, the investigators [7] reported that when activated and stored in nutritionally incomplete substrates, spores die rapidly or undergo deactivation with retention of viability; with activation in a nutritionally complete medium (milk) and storage at subminimum growth temperatures, the spores die more slowly and there is little evidence of deactivation. Heat activation of spores presents some interesting possibilities in connection with the control of spoilage in evaporated milk.[8]

In studies by Curran and Evans [9] suitable additions of soluble iron or manganese salts to fluid organic media increased greatly the total spore yields of three *Bacillus* species. Exposure of milk to rusting utensils stimulated subsequent development of spores comparable in degree to that obtained with purified iron salts. It was concluded that the relationship between iron rust and spore development has practical significance in the commercial canning of milk.

The effect of environmental factors on the response of heated spores should be recognized in investigations on heat resistance of spores. In trials with an organism causing spoilage in evaporated milk, regular results,[10] without dormancy or delayed germination, were obtained when evaporated milk was used as the subculture medium but not when nutrient broth was employed.

Incubation. At one time, cans of evaporated milk often were incubated at the plant from one to several weeks with the idea of giving incompletely sterilized or defective cans an opportunity to spoil so that they could be detected easily. In some instances the incubation was carried out at room temperature; in others a warm room was used to favor activity of any organisms in the milk. Usually, the incubation accomplished its purpose but, occasionally, organisms that had not been killed by the heat treatment developed so slowly spoilage was not evident until the milk had left the plant. At present, freshly packed milk may go rather directly to cars or trucks for transportation to markets.

In the supervision of the product of a plant, cans from each lot of milk sometimes are incubated at a relatively high temperature, such as 98.6 F (37 C), and then examined organoleptically and also culturally. This procedure is very useful, but it should be recognized that in certain outbreaks of spoilage the numbers of cans containing

the causative organism are so small they are missed easily if only a few cans are studied. Incubation of cans also permits checking fat separation, crystal formation, etc.

SPOILAGE DUE TO HEAT-RESISTANT BACTERIA

Formerly, gas formation was the common bacterial defect of evaporated milk, but now coagulation occurs more frequently. Off flavors also are encountered occasionally. The percentage of spoiled cans in a defective lot of evaporated milk is extremely variable, owing to differences in extent of the infection of the milk and to variations in the balance between the heat resistance of the organism and the sterilization exposure. In many cases of spoilage the causative species is present in pure culture and is isolated readily when the milk is cultured under suitable conditions.

Coagulation. Various organisms have caused coagulation of evaporated milk; often the milk also has an abnormal odor and flavor.

The Vermont Agricultural Experiment Station [11] reported an instance of coagulation of evaporated milk in which the change was noted 12 to 24 hr after heating; the milk curdled without souring and then the curd apparently digested, leaving a whey-like liquid above a mass of slimy curd. *B. subtilis* was the organism most actively involved. Ruyle and Sognefest [12] found growth of *B. subtilis* in coagulated evaporated milk was characterized by a change to a gel or custard-like consistency with little or no change in *p*H. Spores of the organism showed unusual heat resistance. Spoilage involved 3 to 5 cans per 1,000 packed.

Hammer [13] studied an outbreak of abnormal evaporated milk in which the typical change was firm coagulation although a very small percentage of the spoiled cans showed a bulging. It was reasonably certain that at least some of the spoiled milk was heated to 236 F for 36 min. May and June milk showed the greatest percentage of spoilage. The period required for coagulation could not be determined, but the heated milk was held at the plant for at least 10 days and was not coagulated then. The spoiled milk had a sweetish, cheesy odor, not at all disagreeable, and a flavor that was faintly sour and cheesy. The acidity was higher than that of unspoiled milk from the same lot. An organism was observed quite regularly in microscopic preparations from the abnormal milk, and when it was cultivated and inoculated into normal cans of milk it produced the typical defect; it was described and named *Bacillus coagulans.* Cordes [14] investigated an out-

break of "flat-sours" in a condensery and reported *B. coagulans* responsible.

An outbreak of coagulation in evaporated milk that occurred during an abnormally hot, dry summer was found to be due to *B. coagulans* by Sarles and Hammer.[15] The percentage of cans that spoiled varied from lot to lot and in one instance was estimated by the plant operators to be 60%. At the condensery, acidity of the normal milk was 0.48%; acidity of the coagulated milk was 1.05%. Odor and flavor of the spoiled milk were distinctly cheesy and acid but not suggestive

Figure 27. Coagulated material from a can of evaporated milk artificially inoculated with *Bacillus coagulans.* Iowa Agr. Exp. Sta. Res. Bul. 19.

of putrefaction. Spoilage began with lots of milk subjected to a heat treatment that had been satisfactory over a long period. With the appearance of the defect the sterilizing temperature was increased somewhat, but coagulation of some of the cans continued.

Three types of spoilage in evaporated milk were investigated by Kelly;[16] the responsible organisms were considered to be atypical forms of *B. cereus, Bacillus simplex,* and *B. megatherium.* An organism that produced a nonacid coagulation in evaporated milk was studied by Morrison and Rettger[10] and regarded as a relatively stable variant of *Bacillus vulgatus.*

B. calidolactis was found by Hussong and Hammer[17] to be the cause of coagulation in evaporated milk held at a relatively high temperature, for example 122 F, or above. The milk came from two condenseries. The organism produced a distinctly acid and slightly cheesy odor and flavor in evaporated milk. *B. calidolactis* probably is of little importance as a cause of spoilage in evaporated milk under practical conditions because of its thermophilic character.

An organism that coagulated evaporated milk in an unusual manner was studied by Hammer and Hussong.[18] It was found in only three of many cans examined in a study of coagulated milk returned to a condensery from various cities, and these were from one lot. The organism curdled inoculated cans of evaporated milk slowly at 98.6 F with no change in the odor or flavor unless incubation was extended; it could be found microscopically for only a short time after inoculation and then only in small numbers. If the inoculated milk was given a good air supply by transferring it to test tubes or removing part of the milk from the can, the organism curdled milk rapidly, produced an objectionable odor and flavor, and could be found in large numbers microscopically. The organism was identified as *B. cereus.* It was not considered the primary cause of coagulation in the outbreak studied since it was isolated from inoculated cans after extended incubation so that it probably had not died out in the milk failing to yield it; also, there was an increase in soluble and amino nitrogen in evaporated milk that coagulated following inoculation with the organism, whereas the soluble and amino nitrogen in the usual milk from the outbreak were the same as in normal milk. Extent of the coagulation in cans returned to the condensery varied from a few small masses of soft curd to complete coagulation. Presumably, an instability in the milk and high holding temperatures were responsible for coagulation in most of the cans.

Bothe [19] examined 36 faulty samples of evaporated milk, characterized by thickening without gas formation, and found two distinct types of coagulation, a "flat-sour" type with *p*H values of 4.7 to 5.4 and a "flat" type with a *p*H range of 5.8 to 6.3. The former defect was associated with the presence of *Bacillus circulans* and the latter with that of *B. subtilis, B. cereus,* and *B. mesentericus.*

Gas Formation. Cans of evaporated milk sometimes undergo a gassy fermentation which results in bulging, particularly at the ends of the cans, and sometimes in bursting along a seam. Coagulation usually accompanies the gas formation and often objectionable odors develop. When bulged but unbroken cans are punctured, the contents may foam through the opening for considerable periods. Because of variations in the condition of the gassy milk, it is evident that the causative organism is not always the same.

The spore-forming anaerobes constitute the important group that produces gas in evaporated milk. These organisms are unusually heat resistant and commonly are responsible when there is a considerable percentage of blown cans in a lot of milk. Various species appear to

be involved and, although most of them produce very objectionable odors, some do not. *Plectridium foetidum* has been identified as a species producing a very foul odor.[20]

Bulging of cans of evaporated milk may result from causes other than bacterial action. Examples are:

1. Filling Cans with Very Cold Milk. When cans are filled with very cold milk and sealed, they may, after processing, show a bulging at ordinary temperatures due to expansion of the contents. Variations in atmospheric pressure may be a contributing factor.

2. Chemical Action. There is a possibility that acid present in evaporated milk may act on the metal and combine with a part of the iron, thus liberating hydrogen, but the condition certainly is unusual.

Bulging that is due to bacterial action is recognized easily by the odor and other changes accompanying gas formation and by bacterial cells in microscopic preparations.

Bitterness. Occasionally, a bitter flavor is encountered in evaporated milk. Sometimes it accompanies coagulation, since certain organisms coagulating milk also digest it with formation of bitter products; in other cases it appears to be the only conspicuous change.

Hammer [21] investigated bitterness in evaporated milk and isolated an organism that produced the condition on inoculation. The first isolation was made from a can showing bitterness and an abnormal odor but no evident coagulation, whereas later the organism was obtained from a can of coagulated milk supplied by the plant reporting the defect. With extended holding, inoculated evaporated milk showed complete coagulation; microscopic examinations then suggested that the organism had largely died out. The organism was named *Bacillus amarus.*

Spitzer and Epple [22] studied bitter evaporated milk in which bitterness was the only objectionable feature. The causative organism was *Bacillus panis.* It rapidly produced large amounts of peptone and lower complex nitrogenous compounds that may have caused the bitterness. The organism was destroyed in 8 to 10 min at 250 F under steam pressure.

Nichols *et al.*[23] isolated 3 organisms from defective canned cream that, on inoculation into normal cream, caused bitterness and thinning. The organisms were strains of *B. subtilis;* their spores withstood temperatures up to 248 F for 40 min. Rate of development of bitterness and thinning in inoculated cans of cream varied with the culture and the incubation temperature. The bitterness and thinning were

associated with breakdown of the protein. Thirty-one strains of aerobic spore formers from normal and spoiled cans of evaporated milk and cream were inoculated into normal cans of the products by Nichols; [3] the outstanding defect induced was a bitter flavor associated with thinness.

SPOILAGE DUE TO DEFECTIVE CANS

An occasional can of evaporated milk spoils as a result of a defect in the container that permitted entrance of organisms. A can may be defective when filled or it may develop a defect during holding. The fermentations that develop in defective cans are of various types and, commonly, are due to mixed floras. Often the flora is made up of nonspore-forming bacteria, which indicates that the organisms entered the product after the heat treatment.

Streptococci frequently are involved in coagulation of the milk in defective cans. Savage and Hunwicke [21] isolated various species belonging to the *Escherichia-Aerobacter* group as well as coccus forms and a yeast. Nichols [3] found that spoilage due to nonspore-forming bacteria consisted chiefly of gas formation and/or curding. Various bacteria were isolated from cans showing the defects, but strains of *Escherichia* were the most common cause of gassy spoilage.

A can of evaporated milk which showed a fishy flavor was investigated by Hammer [25] and an organism capable of producing fishiness in milk, cream, and evaporated milk was isolated. In inoculated milk there was coagulation and rapid digestion in addition to development of a fishy flavor. The organism now is designated *Pseudomonas ichthyosmia.*

Hammer [26] obtained a *Streptococcus,* which was designated *Streptococcus distendens,* from a can of evaporated milk showing slight gas formation. In inoculation experiments it never produced sufficient gas to burst a can; although it grew well in sterile skim milk, gas formation was not noted unless the milk was sealed in some way, for example, with a wax. *S. distendens* was isolated from evaporated milk in which other nonspore-forming organisms also were growing.

In a can of evaporated milk, slight gas development, which might go unnoticed in a tube of milk, makes itself evident because all the gas is held. Inoculation into a can of milk thus affords a convenient method of detecting slight gas formation with organisms that grow in this material.

During World War II spoilage of evaporated milk due to defects in

the containers was relatively extensive. Because of the shortage of tin, a thin coat was used so there was a greater than normal opportunity for defects in it. Also, it often was necessary to handle and hold the milk under adverse conditions.

CONTROL OF SPOILAGE

Control of spoilage in evaporated milk that definitely is due to an organism surviving the heat treatment, and not to some such irregularity as defective containers, requires answers to a series of questions, including the following: (1) Has there been an unintentional decrease in the sterilization exposure due to variations in thermometers or some other cause? (2) Has the quality of the milk received at the plant been maintained when warm weather, or some other factor, made quality control more difficult? (3) Have pipe lines or other equipment been given less complete cleaning and sterilization,[8] or has concentrated or unconcentrated milk been held under conditions favoring growth of heat-resistant organisms, thus permitting a build up of types difficult to kill? The high heat resistance of certain spoilage organisms grown at about their optimum temperature should be recognized in operating a plant putting out a product intended to be sterile. "Cut-back" milk held over should be properly refrigerated. (4) Are the opened cans of spoiled milk being so handled that all danger of contamination of equipment, milk, cans, etc., with organisms in the product is avoided? The organisms can be carried on the hands and clothing of employees, by flies and insects generally, and in various other ways.

As soon as an outbreak of spoilage develops, all possible precautions should be instituted, without waiting for a detailed analysis of the problem. Eventually, a thorough review of the processing during the period of spoilage may indicate the probable cause of the irregularity. There is some evidence that the raw milk, or other conditions, in a given area may make it especially difficult to produce evaporated milk that keeps properly, even when special care is used in controlling the bacteriological condition of the product.

AVOSET AND CANNED FRESH MILK

Avoset. Avoset is a stabilized cream that is packaged in bottles after sterilization. Fresh cream,[27] with sodium alginate added, is sterilized in a continuous flow by direct application of high-pressure

steam. In the first units of the equipment the cream is preheated, under pressure, in 2 kettles. It next flows to the sterilizers and is processed at 260 to 280 F. The sterilization period may vary but seldom is over 4 min. The cream then flows to the homogenizer, where the pressure is kept below 2,000 lb in the first stage and below 500 lb in the second. Temperature of homogenization is not critical but should be above 150 F, whereas pressure must be gauged carefully to avoid too much viscosity in the product. From the homogenizer the cream flows into cooling coils and then into sterile holding tanks that feed the bottle filler. In the bottling room radiations from ultraviolet lamps guard against product contamination. Operators wear sterilized uniforms, gloves, and masks. Bottle caps first are loaded into filling magazines, then placed with bottles in retorts and steam sterilized at 275 F. The bottles are placed on an endless conveyor, protected by a bank of bactericidal lamps, which takes them through the filling and capping machines that perform sterile operations. The entire room is scrubbed and steamed regularly. Air entering the bottling room is specially cleaned and conditioned. After capping, the bottles travel over an air-cooled conveyer which removes most of the heat from them. Laboratory tests establish the bacteriological and physical qualities of the product.

Canned Fresh Milk. Fresh milk can be processed in the same way as evaporated milk, but special procedures also have been developed for canning it.

With the Graves-Stambaugh process,[28] fresh milk is collected under vacuum in 100 to 200 gal stainless steel farm tanks. At the plant the milk is heated to 120 F, homogenized at 2,000 lb pressure, and forced through heat exchangers where the temperature is raised to 285 F in <5 sec. Heating is with steam at 80 lb pressure. The milk is held at 285 F for 8 sec, cooled to 110 F in a second pair of heat exchangers, and canned with an aseptic canning machine. The product is reported to keep 4 to 6 months without refrigeration.

Martin [29] noted that fresh milk can be sterilized in a continuous heat exchanger and immediately canned aseptically, the filling and sealing taking place in an atmosphere of steam; the product can be kept 6 to 9 months without refrigeration.

In the Winger process,[30] milk is handled in the usual manner at the plant except that edible hydrogen peroxide is added at a certain point in the processing and after 30 min removed with an enzyme. The milk goes through the homogenizer and then is cooled to room temperature, placed in cans, and sterilized for 30 min at a temperature

above boiling. In experiments, milk with 5,000,000 bacteria per ml was reduced to 30,000 with the peroxide treatment and then to 0 by the heating. The product is reported to keep indefinitely without refrigeration; if stored at 120 F, or higher, the fat melts and rises to the top as an oily layer.

REFERENCES

1. *J. Med. Research,* **39,** 349 (1919).
2. *Arch. Pediat.,* **48,** 42 (1931).
3. *J. Dairy Research,* **10,** 231 (1939).
4. *Ia. Agr. Expt. Sta. Research Bull.* 244 (1938).
5. *J. Infectious Diseases,* **56,** 196 (1935).
6. *J. Bacteriol.,* **49,** 335 (1945).
7. *J. Bacteriol.,* **53,** 103 (1947).
8. *Food Inds.,* **20,** 370 (1948).
9. *J. Bacteriol.,* **67,** 489 (1954).
10. *J. Bacteriol.,* **20,** 299 (1930).
11. *Vt. Agr. Expt. Sta. Bull.* 170 (1912).
12. *J. Milk and Food Technol.,* **14,** 173 (1951).
13. *Ia. Agr. Expt. Sta. Research Bull.* 19 (1915).
14. *J. Dairy Sci.,* **11,** 46 (1928).
15. *J. Bacteriol.,* **23,** 301 (1932).
16. *Trans. Roy. Soc. Can.* V, 387 (1926).
17. *Ia. State Coll. J. Sci.,* **6,** 89 (1931).
18. *J. Dairy Sci.,* **15,** 220 (1932).
19. *Deut. Lebensm. Rundschau,* **47,** 183, 197, 232 (1951).
20. Hunziker, O. F., *Condensed Milk and Milk Powder,* 7th ed. 1949. Published by the Author, La Grange, Ill.
21. *Ia. Agr. Expt. Sta. Research Bull.* 52 (1919).
22. *J. Dairy Sci.,* **3,** 486 (1920).
23. *J. Dairy Research,* **8,** 331 (1937).
24. *Food Inspection Bd. Great Britain, Spec. Rept.* 13 (1923).
25. *Ia. Agr. Expt. Sta. Research Bull.* 38 (1917).
26. *Ia. State Coll. J. Sci.,* **2,** 1 (1927).
27. *Food Inds.,* **17,** 875 (1945).
28. *Food Eng.,* **23,** 6, 71 (1951).
29. *Chem. Eng. News,* **30,** 538 (1952).
30. *Butter, Cheese, Milk Products J.,* **43** (2), 30 (1952).

12 Bacteriology of Sweetened Condensed Milk

Whole or skim milk sometimes is converted to a sweetened condensed product and put in tins for use in homes, and in cans and barrels for use in bakeries, candy factories, ice cream plants, etc.; barrels may be produced during periods of surplus milk for convenient storage. Keeping qualities of the product depend primarily on the concentration of added sucrose or, occasionally, other sugar and to some extent on the concentration of milk solids; destruction of organisms during the processing also is a factor. The plasmolyzing action of the materials in solution effectively prevents development of most organisms. This is shown by the spoilage of diluted sweetened condensed milk under conditions in which the undiluted product shows no change. Sweetened condensed milk keeps for considerable periods when allowed to stand open at temperatures satisfactory for growth of many micro-organisms, and this is one of its important advantages in areas with limited refrigeration facilities.

Microbiological Examination. If a can of milk is to be cultured, it must be opened with care to avoid contamination; the procedure used with evaporated milk is satisfactory. Collection of samples from barrels also requires care if contamination is to be avoided. Ordinarily, cultures should be made in bouillon or on agar containing large amounts of sucrose (30 to 40%) to simulate the high osmotic pres-

sure in sweetened condensed milk; because organisms normally are present, plate counts on agar containing considerable sucrose frequently are desirable.

In investigations on microbiological spoilage in sweetened condensed milk, microscopic examination of smears made from the diluted product (for example, 1 part to 5 parts distilled water) may reveal useful information, such as the predominant organisms, actively budding yeast cells, etc. With certain stains and careful observation, some dead bacterial cells and leucocytes commonly can be seen.

Since various organisms can be present in sweetened condensed milk in relatively large numbers without affecting it appreciably, well-controlled tests are necessary in establishing a particular organism as the cause of a defect.

Importance of Sugar Concentration. Practical experience has shown repeatedly that with low sugar concentrations spoilage is common. Rice and Downs [1] investigated the importance of sugar concentration in the keeping qualities of sweetened condensed milk. The percentage of sugar in solution in the water was calculated from the percentages of sugar and water and was designated the sugar ratio; for example, milk with a sucrose content of 46.6% and a moisture content of 27.4 has a sugar ratio of 63.0 [46.6 ÷ (46.6 + 27.4) = approximately 0.63 or 63%]. In order definitely to prevent growth of an organism causing thickening in sweetened condensed milk, it was necessary that the sugar ratio be at least 64.5. As the ratio fell below this, the organism became more and more active until at 63.0 a strong odor developed and solidification began.

An organism isolated from gassy sweetened condensed milk grew in saturated sucrose bouillon but failed to produce gas in one of the brands of sweetened condensed milk into which it was inoculated.[2] This suggests that the milk solids may be a factor in restraining growth as well as the sugar, especially since the brand in which the organism would not form gas was one high in milk solids.

Some lots of sweetened condensed milk keep with a sugar ratio lower than the one suggested by Rice and Downs.[1] Low holding temperatures favor keeping; the types of organisms present also are a factor and these can be greatly influenced by the care used in selecting the original milk and in processing it. Excessive sugar ratios are avoided since they may lead to sucrose crystallization when the finished product is held at low temperatures.

Normal Sweetened Condensed Milk. Various investigators have noted that sweetened condensed milk lacks sterility, and a few have

reported the numbers of organisms found, which vary from <100 to 100,000 or more per ml. It is probable that the quality of the materials employed and the care used in handling are important factors in determining the numbers. Cocci of various types probably have been the most common organisms reported but aerobic spore-formers also have been found.[3,4] Downs [5] stated that the bacterial count of sweetened condensed milk, determined by the plate method shortly after its manufacture, is a good indication of the sanitary conditions in the plant producing it.

Coutts [6] considered the growth of bacteria in sweetened condensed milk and referred to previous studies showing increases may occur. Andrews [7] found that *M. pyogenes* var. *aureus* increased greatly, and his experiments suggest that condensed milk is almost a differential medium for growth of micrococci.

It is evident that sweetened condensed milk contains organisms and these may undergo some growth and the product still remain normal. How extensive the growth can be without the product becoming abnormal presumably depends on the types of organisms involved and perhaps other factors. General laboratory experience indicates that micrococci are the organisms most often multiplying in the normal product and that the holding temperature definitely influences the rate of multiplication; apparently, several hundred thousand of the organisms per milliliter sometimes are present without a conspicuous defect being noted. Extensive increases in numbers usually are followed by decreases which may be rather rapid.

MICROBIOLOGICAL SPOILAGE

Gas Formation. Gas sometimes develops in sweetened condensed milk and may result in bulging and blowing of cans and leakage and bursting of barrels. In certain instances, the fermentation has been very serious because it appeared suddenly and involved practically the entire pack for a number of days. The organisms usually responsible for development of gas in sweetened condensed milk are yeasts that ferment sucrose but not lactose.

Pethybridge [8] isolated two types of yeasts from gassy sweetened condensed milk put up at an Irish condensery. Each type formed gas in sweetened condensed milk and in saturated sucrose bouillon but did not ferment lactose. The predominant yeast was oval and the other spherical. Yeasts were obtained from gassy sweetened con-

densed milk by Hunziker.[9] In sucrose bouillon they caused violent gas formation.

Hammer [2] isolated an organism which has been named *Torulopsis lactis-condensi*. It was isolated from a very serious outbreak of blown milk involving about 10 lots packed at one plant; 7 to 8 days elapsed between canning the milk and development of pronounced swelling. The defect largely, but not entirely, involved milk that had passed

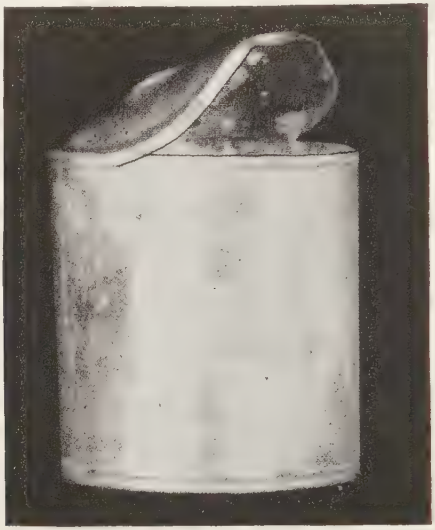


Figure 28. Can of sweetened condensed milk blown open after artificial inoculation with *Torulopsis lactis-condensi*. Iowa Agr. Exp. Sta. Res. Bul. 54.

through a certain filler in which it was more exposed than in the other filler used in the plant. Development of gas was so extensive that the cans were bulged greatly and often ruptured along the seams. When swollen but unbroken cans were punctured, milk and large volumes of gas foamed through the opening for a considerable period. The fermented milk had a yeasty flavor but was not disagreeable, and there was no suggestion of putrefaction. A yeast was evident microscopically and was isolated readily by plating the material on beef extract agar to which 30% sucrose had been added. Inoculation trials with one brand of sweetened condensed milk gave only negative results, but with another brand gas formation usually resulted. The organism grew well in bouillon to which 30% sucrose had been added and produced considerable alcohol in the medium; it also grew in

bouillon saturated with sucrose. Results were obtained which indicate that yeasts capable of growth in 30% sucrose bouillon can be isolated rather commonly but that most of them cannot produce gas in sweetened condensed milk.

Two types of yeasts were isolated from gassy sweetened condensed milk by Knudsen;[10] Danish milk yielded only a spherical type but from Dutch milk both a spherical and an oval type were obtained. Knudsen assumed the yeasts were identical with those found by Pethybridge. Hiscox[11] obtained two yeasts capable of producing gas in milk containing 70% sucrose and in sweetened condensed milk. They were identical with the organisms isolated by Knudsen.

Olson and Hammer[12] studied 6 samples from an outbreak of gassiness in sweetened condensed milk that was marketed in barrels. Development of gas occurred more or less frequently in the milk packed during several summer months. Most of the defective lots did not develop gas until some time after they had left the plant. All the samples yielded an oval yeast and one of them also contained a spherical type. Two samples of gassy milk from another source yielded the oval yeast and one of them also contained a spherical yeast. The oval type was identified as *T. lactis-condensi.* The name *Torulopsis globosa* has been suggested for the spherical type which apparently is identical with the spherical yeast isolated from gassy milk by various investigators. The authors concluded from their studies and reports on previous investigations that gas formation in sweetened condensed milk commonly is due to *T. lactis-condensi* or to *T. lactis-condensi* and *T. globosa* growing together, and that, when the two species are together, *T. lactis-condensi* is the more numerous. They noted, however, that Knudsen[10] reported only the spherical type in a sample of Danish blown milk.

Rogers and Clemmer[13] found an *Escherichia-Aerobacter* organism producing gas in sweetened condensed milk containing 40 to 45% sucrose. The temperature of the milk in the forewarmer was high enough to destroy the organism, but inspection of the plant experiencing the defect showed abundant opportunity for infection, particularly by flies, between the pan and the can filler.

The available data indicate that the organisms generally responsible for gas formation in sweetened condensed milk are not sufficiently resistant to survive the heat employed in the manufacture, so that contamination after the condensing must be the cause of the abnormality. Contamination probably most often comes from the plant equipment. In one outbreak[9] the crust of sugar on the inside of the

chute carrying sugar from the floor above contained practically a pure culture of the yeast present in the spoiled milk.

Thickening. Sweetened condensed milk sometimes undergoes thickening. In the early stages only portions of the milk in a can may be involved, but later the milk may be thick throughout and pour with difficulty. Thickening sometimes is the result of a physical change that is influenced by various factors, such as season of production, high storage temperature, long holding, acidity, salt balance, etc., but also can be due to action of organisms. With continued growth, the organisms causing thickening usually produce an abnormal flavor.

Rice and Downs [1] studied bacterial thickening of sweetened condensed milk and found it due to cocci that are capable of inverting and destroying sucrose and producing considerable acid. They grow in fairly high sucrose concentrations but can be checked if the concentration is sufficiently high; at temperatures above room temperature they are particularly active. The organisms were isolated from factory equipment which suggests that contamination of the milk may come from this source.

Downs [5] reported that the organisms thickening sweetened condensed milk belong to one general type of *Micrococcus* of which two distinct varieties were noted. Many organisms of common occurrence closely resembled them and could only be distinguished from them by their effect on sweetened condensed milk. Downs considered that the thickening is due to production of a rennin-like enzyme; the nonimportance of acid is shown by the failure of certain organisms producing a high acidity in the milk to thicken it and also by the sweet curdling of litmus milk in which the organisms causing thickening have grown. Exposure to 147.2 F for 10 min killed the organisms in broth.

Development of Molds. Molds may grow in cans of sweetened condensed milk, either on the surface of the milk or on milk adhering to the side or top of the can. Some molds form colonies without any significant effect on the milk; these may be conspicuous, or they may be very inconspicuous and consist only of a small mycelial growth and a few spore heads. Other molds coagulate the milk in the areas of the colonies to form masses that are referred to as buttons. They may lie free in the milk, so that they can be lifted easily, or they may be attached rather firmly to the side or top of the can. Apparently, a button may continue to increase in size long after the mold ceases growth, due to diffusion of the mold protease. Milk near mold growth

often has a brownish color and objectionable odor and flavor. Buttons sometimes are conspicuously brown.

Molds grow rather slowly in cans of sweetened condensed milk, even at favorable temperatures, presumably because of the limited oxygen supply. Growth is most conspicuous when the cans are left in one position for an extended period. If the cans are inverted occasionally, growth tends to be less extensive and some of the buttons may be imbedded in the mass of milk so that they are not seen easily. Presumably, many buttons in sweetened condensed milk are missed by the consumers because of the manner of opening the can or using the contents.

Figure 29. Buttons on sweetened condensed milk. Courtesy A. M. Besemer.

In underfilled cans mold growth may be very conspicuous due to the increased air supply. It appears that relatively rapid and extensive growth of coccus forms in sweetened condensed milk tends to limit, or entirely prevent, growth of molds, presumably by quickly using the oxygen in a can.

Rogers *et al.*[14] found that buttons are due to growth of *Aspergillus repens* and possibly other molds and that using the air in a can prevents continued development of the mold. They suggested that buttons can be prevented by keeping out the molds through careful methods or by making the conditions unsatisfactory for their growth, either by use of a low holding temperature or by limiting the oxygen. The investigators noted that buttons did not develop in milk held at 68 F and that sealing the cans under a vacuum of 20 in., or more, prevented their formation.

Knudsen[10] reported that in Europe buttons in sweetened condensed milk often were caused by *Aspergillus glaucus;* another mold, *Catenularia fuliginea,* forms somewhat similar buttons. About 40 cans of milk from a factory that had encountered buttons were examined by Nichols[15] who found that 77% of the cans, sampled at random from 15 days' manufacture, showed buttons. Three species of organisms were isolated, one belonging to the genus *Actinomyces* and two to the genus *Penicillium.* Czulak and Hammond[16] encountered tins of milk in which black spots appeared on the ends after a few

weeks at room temperature; these later developed into buttons. The mold was considered to be a species of *Cladosporium*.

Other Defects. A fruity flavor sometimes is encountered in sweetened condensed milk. It varies in degree and, when conspicuous, may be accompanied by lumps of thickened milk. Presumably, the condition is due to coccus forms that succeed in growing rather extensively in the product.

Nichols [15] studied a can of sweetened condensed milk that had a putrid flavor, suggestive of bacterial proteolysis, and was considerably thinned; the plate count was 267,000 per ml and a positive presumptive coliform test was obtained with 0.1-ml amounts. Nichols also encountered cans that lacked the freshness of flavor which is usually characteristic of sweetened condensed milk.

Control of Microbiological Defects. Since certain bacteria, yeasts, and molds cause defects in sweetened condensed milk, the numbers of these organisms in the product should be kept to a minimum by adequate precautions with the original milk supply, the heat treatment, equipment care, sugar supply, cans, air contamination, etc.

Recent studies suggest the necessity of adequate cleaning and care of equipment.[17] Crossley [4] considered that the final can filling and sealing contributes the majority of the micrococci, yeasts, and molds to the milk and noted that small, but significant, numbers of organisms may be added by the cans and lids.

In the control of mold growth in sweetened condensed milk, an important consideration is treatment of the air entering the canning room to reduce greatly the numbers of organisms in it. Use of cans that are practically full when sealed limits the air supply of any molds present in the milk and appears to be a useful procedure. Cans and lids can be flamed just before use. Crossley [1] reported that inversion of cases of milk 4 to 6 times at 10- to 14-day intervals was sufficient to reduce mold deterioration.

ENZYMATIC AND CHEMICAL CHANGES

Both enzymatic and chemical action may be involved in the deterioration of sweetened condensed milk. Results of Rice and Markley [18] indicate that rancidity is due to the action of milk lipase, and Rice [19] reported data supporting this conclusion. The enzyme hydrolyzes the milk fat and liberates fatty acids, to some of which the rancid flavor is due. Addition of only 0.75% unheated milk to a batch of sweetened condensed milk may cause rancidity; [19] this is equiva-

lent to 0.3% of the amount of whole milk from which the lot was prepared. Addition of pancreatic lipase produced the same result, but the condition was not produced by the organisms used. Heating whole milk to 140 F for 15 min, or to 150 F for an instant, destroyed the lipolytic power, but when sugar was present, or when condensed milk was heated, a higher exposure was required. Leaky intake valves and carelessness in heating the milk in the hot wells are factors that may result in the presence of active lipase in the finished milk.

Crossley [4] noted that development of certain off flavors in sweetened condensed milk appeared to be due to activity of lipolytic and proteolytic enzymes of microbial origin, the enzyme action continuing after the organisms were no longer viable.

Tallowiness in sweetened condensed milk was investigated by Rice,[20] who pointed out that it is due to action of the oxygen of the air on the butterfat, the reaction ordinarily being catalyzed by small amounts of copper. Biological agencies were excluded as a cause of tallowiness because (1) the condition apparently developed at 32 F as rapidly as at room temperature, (2) neither heat sterilization nor strong preservatives prevented it, and (3) bacterial counts on tallowy samples were low. Tin did not catalyze the reaction, whereas iron did to a much less degree than copper.

REFERENCES

1. *J. Dairy Sci.*, **6**, 532 (1923).
2. *Ia. Agr. Expt. Sta. Research Bull.* 54 (1919).
3. *J. Med. Research*, **39**, 349 (1919).
4. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 438 (1949).
5. *J. Dairy Sci.*, **8**, 344 (1925).
6. *Rept. Local Gov. Board Pub. Health Med. Subj. Food Rept.* 15 (1911).
7. *J. Pathol. Bacteriol.*, **18**, 169 (1913).
8. *Econ. Proc. Roy. Dublin Soc.*, **1**, 306 (1906).
9. Hunziker, O. F., *Condensed Milk and Milk Powder* (7th ed.), 352, 1949. Published by the Author, La Grange, Ill.
10. *K. Veterinaer-og Landbohøskole Aarsskrift*, 282 (1922).
11. *Ann. Appl. Biol.*, **10**, 370 (1923).
12. *Ia. State Coll. J. Sci.*, **10**, 37 (1935).
13. *Abstr. Bacteriol.*, **2**, 6 (1918).
14. *J. Dairy Sci.*, **3**, 122 (1920).
15. *J. Dairy Research*, **10**, 231 (1939).
16. *Australian J. Dairy Technol.*, **8**, 66 (1953).
17. *Proc. Soc. Appl. Bacteriol.* (1), 1 (1947).
18. *J. Dairy Sci.*, **5**, 64 (1922).
19. *J. Dairy Sci.*, **9**, 293 (1926).
20. *J. Dairy Sci.*, **9**, 459 (1926).

Bacteriology of Milk Powder

13

In the preparation of milk powder or dry milk, the reduction in bulk for purposes of storage or transportation is carried to the extreme since there is only a small percentage of moisture in the finished product. Milk powder antedates both evaporated milk and sweetened condensed milk, its history going back to medieval times when it was prepared by very simple procedures. At present, milk is dried by the roller process (atmospheric or vacuum) and also in various spray systems. Both whole milk and skim milk are dried. The latter product (nonfat dry milk solids) sometimes is spray dried after relatively low exposure to heat and used in making culture buttermilk, cottage cheese, etc. Dry skim milk also is marketed extensively in small packages for reconstitution and other uses in the home. Milk powder in tight containers sometimes is packed in an inert gas to delay deterioration.

The bacteriology of milk powder is relatively simple because (1) the usual manufacturing procedures result in destruction of many of the organisms in the original milk or added from various sources, and (2) the low moisture content prevents growth of the organisms that survive the processing and is an important factor in causing a decrease in the numbers of those in the fresh powder. The primary problems presented by the product are in connection with its deterioration and reconstitution, and these are essentially chemical in nature.

Determination of Numbers of Bacteria. Both the plate and microscopic [1] methods have been adapted to the examination of reconstituted milk powder. Ordinarily, a relatively large sample, such as 10 or 11 g, is more representative than a 1-g sample because of the variations from one point to another in a container. Results of bacterial counts are calculated to a 1-g basis or to 1 ml of reconstituted milk; the 1-ml calculation simplifies comparing the counts with counts on market milk.

Plate Count. Methods of making agar plate counts on milk powder have been studied by a number of investigators,[2-9] largely because of the difficulty in dispersing properly certain lots of powder. Without proper dispersion it sometimes is difficult to distinguish between powder particles and small colonies. Warm dilution blanks, dilution blanks made with various reagents, and beads in the blanks all have been advocated to aid in dispersion. Dilution blanks of 1.25% sodium citrate solution and containing beads normally are very satisfactory; with powders that are difficult to disperse, the blanks can be warmed to 104 to 113 F (40 to 45 C).

Thiel and Pont [6] reported that concentrations of lithium and sodium hydroxides which effected complete dispersion of milk powder appreciably reduced plate counts. With 3.5% sodium citrate in tap water, a clear plate was obtained even with a 1 to 100 dilution, and there was no reduction in plate counts in tests on fresh powder. Preliminary warming of blanks to 104 F (40 C) was necessary to disperse roller powder. Garrison,[7] using dilution blanks warmed to 113 F (45 C) and containing beads, obtained best results on milk powders with 1.25% sodium citrate solution since this usually completely dispersed the powder and showed no evidence of being bactericidal. A solution of 0.075% lithium hydroxide definitely was bactericidal. Cone and Ashworth [8] reported that reconstituting spray-dried whole milk powder in water at 113, 122, or 131 F (45, 50, or 55 C) gave much higher plate counts than water at 71.6 to 77 F (22 to 25 C), with 122 F appearing to give slightly higher counts than 113 or 130 F. Reconstituting with 0.1 *N* lithium hydroxide significantly lowered the counts. Solutions of sodium citrate (0.5 to 1.25%) or disodium phosphate (1.25%) appeared to interfere slightly with heat activation of the cells, and 1/60 *N* sodium hydroxide markedly interfered with it. None of these solutions exhibited any toxicity at room temperature. White [9] found that reconstitution of spray- and roller-dried milk in water at 122 F (50 C) gave higher plate counts than reconstitution at 104 F (40 C). He also noted that contact with

0.1 *N* lithium hydroxide for 15 min may destroy 99 to 100% of the organisms in dried milk.

Microscopic Count. Milk powder for direct counts can be dispersed advantageously in 1.25% sodium citrate solution. With most of the organisms dead as a result of the heat exposure, proper staining and good lighting are necessary in making the counts.

Supplee and Ashbaugh [10] mixed milk powder with enough water to give the solids concentration in natural milk and made microscopic counts. With milk dried on an atmospheric roll, the method showed from 30 to 78% (average 45.3) of the organisms present in the original milk. There was a tendency for the groups of bacteria existing in natural milk to be broken during the drying. Jephcott *et al.*[11] found relatively few bacteria in the microscopic examination of roller powder and concluded that the majority of the organisms actually are destroyed or rendered unstainable in the drying.

North [12] regularly obtained higher microscopic counts on milk powder smears stained with aniline oil methylene blue * than with plain methylene blue. With 30 (88.2%) of 34 samples, the counts were at least twice as high, with 12 (35.3%) they were at least 5 times as high, and with 3 (8.8%) they were at least 10 times as high. When smears stained with plain methylene blue were decolorized and restained with aniline oil methylene blue, the counts on 26 (76.5%) were more than doubled, on 11 (32.4%) they were at least 5 times as high, and on 3 (8.8%) they were 10 times as high. With raw milk there was no significant difference between the counts obtained with the two stains. North noted that the aniline oil stain has the definite advantage of producing a pale blue background against which the organisms are deeply stained, there is little or no chance of overstaining, and the smears require no special handling but may be transferred from xylene to alcohol and then to the stain with no intermediate steps.

Results of various laboratories indicate that proper staining is needed for microscopic counts on milk powder and that careful observations are necessary. Even with the best technic not all the organisms in milk powder as a result of contamination from various sources can be seen in preparations from it; presumably, the heat treatment is largely responsible. The percentage of the total organisms that can be seen apparently varies with the types of organisms, heat treatment, staining procedure, etc.

* Aniline oil 3 ml, ethyl alcohol (95%) 10 ml, 1.5 ml concentrated hydrochloric acid added slowly to the aniline oil and ethyl alcohol, 30 ml saturated alcohol solution of methylene blue, and water to equal 100 ml.

Tests for Special Organisms. Milk powder may be examined for thermophiles by incubating plates at 131 F (55 C). Such an examination is intended to detect organisms that may have grown during the holding of milk or concentrate at relatively high temperatures for excessive periods. Examinations also may be made for coliform organisms with the special media employed for this group,[1] the idea being that coliforms indicate inadequate heat treatment or recontamination. Sorensen [13] obtained a thermophilic spore count on skim-milk powder by autoclaving a 1 to 10 dilution of the powder at 5-lb steam pressure for 10 min and plating, with incubation of the plates at 131 F (55 C).

Numbers of Organisms in Milk Powder. The numbers of organisms in milk powder vary widely with either the plate or microscopic procedure. Such variation is due to quality of the milk used or perhaps types of bacteria in it, heat treatment employed, and control of growth and contamination during processing. Bacterial standards for milk powder have stimulated studies on sources of the organisms in it and methods of control. As a result, bacterial counts on powder have decreased during recent years.

Plate Counts. The plate counts reported vary from a few hundred to several million per gram. The effect of the heat treatment used in processing on the bacterial count of the finished powder is shown by the data of Downs.[14] Ten samples of spray-dried powder, manufactured from pasteurized and condensed milk, contained 15,600 to 595,000 bacteria per g whereas 9 samples of spray-dried powder, manufactured from milk not previously condensed, contained 1,500,000 to 3,000,000. Various investigators have noted that counts on spray-dried powder are higher than those on roller-dried powder, other conditions being similar. Macy [15] obtained counts from 4,400 to 5,500,000 on 31 samples of spray-dried powder and from 40 to 7,900 on 13 samples of roller-dried powder. Higgenbottom [16] noted that spray powder gave 3 times as many positive presumptive coliform tests as roller powder.

In the handling of powder some organisms commonly are added to it from equipment, air, etc., the number being determined largely by the care used. Supplee and Ashbaugh [17] obtained counts of 50 to 1,100 per g (average 563) on milk from the cylinder, counts of 300 to 3,000 (average 1,614) after sifting, and counts of 500 to 5,200 (average 3,271) after packing. On a percentage basis, contamination of milk powder during its preparation for market may be considerable but can be kept very low if the possibilities of contamination are recognized and avoided.

Microscopic Counts. On 33 samples of milk powder, North [12] reported microscopic counts that ranged from 1,890,000 to 2,160,000,000 per g with the aniline oil methylene blue stain; 2 counts were over 1 billion, 16 additional were over 100 million, 12 additional were over 10 million, and only 3 were under 10 million.

Results secured in various laboratories show that high microscopic counts are obtained on some lots of milk powder, even when the plate counts are very low. Although the percentage of the bacterial cells originally present that can be stained after drying varies widely, even with the same processing procedure, microscopic counts on milk powder show general differences in bacteriological quality of the product going to the drier. It should be recognized that the milk as received from farms is only one factor in determining this quality; the milk, or the concentrate from it, may be so held in the plant that bacteria multiply extensively, serious contamination may occur, etc.

Bacterial Standards. Bacterial standards have been established by the American Dry Milk Institute for various grades of milk powder, the estimates to be made according to Standard Methods.[1] These are as follows:

Bacterial Standards for Milk Powder

Type and Grade of Powder	Bacterial Estimate per Gram Not More Than
Skim milk powder (nonfat dry milk solids)	
Extra grade	
Spray process	50,000
Vacuum drum	50,000
Atmospheric roller	50,000
Standard grade	
Spray process	100,000
Vacuum drum	100,000
Atmospheric roller	100,000
Whole milk powder	
Gas packed, spray process	
Premium grade	50,000
Extra grade	50,000
Bulk, spray process	
Extra grade	50,000
Standard grade	125,000
Bulk, roller process	
Extra grade	50,000
Standard grade	125,000

There are other requirements of bacteriological significance that must be met, such as cooling of the fresh milk, its reduction time with methylene blue, condition of the cows, etc.

Types of Organisms in Milk Powder. The organisms in milk powder include (1) heat resistant types that have survived the processing and (2) others that represent contamination during part of the processing and also during handling of the powder. Often relatively few species are present.

Various investigators have found streptococci,[5,18] especially *S. thermophilus* and *S. durans,* in comparatively large numbers. Micrococci, sarcinae, and spore formers (*Bacillus* and *Clostridium* types) also have been reported.[5,18,19] With spray powder, streptococci appear to predominate, whereas, with roller powder, micrococci, sarcinae, and spore formers are most common.[6,16]

Mattick *et al.*[19] found the flora of spray powder included streptococci, micrococci, and spore formers. Improvements in plant cleaning resulted in a decline in the relative numbers of streptococci. With a preheating temperature of 165 F and a clean plant, micrococci predominated, whereas, with a preheating temperature of 190 F, streptococci predominated and spore formers were relatively numerous.

Coliform organisms have been found in powder by various investigators [6] but not in large numbers. Crossley and Johnson [5] examined 198 coliform strains from powder and 164 strains from raw milk. *E. coli* types occurred less frequently in the powder, whereas *Aerobacter* strains, especially *A. cloacae,* were most frequent; 48.5% of the strains from powder were heat resistant compared to 2.2% of the strains from raw milk. The coliform flora of powder was due partly to plant contamination by heat-resistant strains, although some non-heat-resistant strains survived spray drying.

Yeasts and molds have been encountered in samples of powder but, commonly, only in small numbers. Higginbottom [16] noted that molds are not a reliable indication of contamination subsequent to drying.

Changes in Numbers of Organisms. Because the low moisture content makes conditions so unfavorable, a decrease in the bacterial content of milk powder during holding would be expected. Supplee and Ashbaugh [17] concluded that the bacteria in milk powder die off rapidly during storage and that, in normal milk made by the roller process studied, the numbers are approximately constant after 2 to 4 months. Powder to which organisms were added showed a similar decrease, but a longer period was required to reach an approximately constant number. Macy [15] reported a remarkable decrease in counts

with both spray and roller powders after 1 yr. The decrease continued and the maximum reduction in counts (99.9%) for the spray milk occurred after 5 yr, whereas the maximum (97.9%) for the roller powder was reached after 3 yr.

Nichols [18] noted that during prolonged storage the viable bacteria in milk powder died. The mean percentage reduction in counts after 12 months at room temperature was 95%. The bacteria tended to die more quickly as storage temperatures increased, and the numbers decreased faster in an atmosphere of air than in one of nitrogen. A variable decline in numbers of bacteria in powder during storage, with some species dying more rapidly than others, was reported by Crossley and Johnson.[5] Spore-forming bacteria survived longest and coliform organisms disappeared rapidly. Higginbottom [20] found with spray- and roller-dried milk, and also whey, that there was a general tendency for the plate counts to fall during storage but that many samples showed little change in count after 6 months or longer. There was a greater tendency for a decrease in count with roller-dried than with spray-dried products, and the decrease was more definite with whey than with milk. Thiel and Pont [6] noted a decrease in total count on all lots of powder after storage, the decrease being more pronounced at the higher temperatures; the thermophilic organisms did not show the same tendency, except when large numbers were present.

Experience in various control laboratories indicates that the decrease in plate counts on milk powder during storage often is pronounced, particularly with a high initial count and relatively high temperatures. Packing in an inert gas tends to delay the decrease.

Effect of Relative Humidity on Organisms. Higginbottom [21] stored two lots of spray-dried milk powder, one from skim milk and one from whole milk, at 77 F for 48 and 103 weeks, respectively, at relative humidities from 0 to 100%. At high relative humidities (80 to 100%) a rapid fall in numbers of bacteria was followed by rapid growth of bacteria and by overgrowth with molds. At relative humidities below 80%, the number of surviving bacteria increased with decreasing humidity to maximum survival at about 10% relative humidity and then tended to fall towards 0%. Maximum survival of bacteria at 5 to 15% relative humidity was confirmed with pure cultures dried in milk. Bacterial spores in milk powder stored over phosphorus pentoxide or concentrated sulfuric acid showed a marked loss of vitality.

Significance of Bacterial Counts. Low plate counts on milk powder normally indicate thorough heat treatment during the processing and

adequate protection against contamination. They give no indication of the bacteriological quality of the original milk because of the great destruction of organisms with normal processing.

Findlay *et al.*[22] found that, with a raw milk supply of poor bacteriological quality, preheating temperatures of 200 and 190 F greatly reduced the bacterial count in the preheated and condensed milk; reduction in count was less marked with 180 F and very considerably less with 170 and 160 F. Bacterial counts on spray-dried powder were low with preheating temperatures of 200 and 190 F; with 180 F they were only slightly higher, but with 170 and 160 F they were relatively high.

Nichols [18] noted wide variations in the mean plate counts of powder from different plants and they seemed to be associated with the technic of manufacture. There was seasonal variation only in the plants in which the general bacteriological quality of the powder was poor. Mattick *et al.*[19] reported that the factors which determine the plate count of milk powder appeared to be, in order of importance: (1) Plant cleanliness and sterility, (2) preheating temperatures, and (3) bacteriological quality of the raw milk, which in turn affected the hygienic condition of the plant. According to Crossley and Johnson,[5] plate counts on milk powder and incidence of coliform organisms showed a variable increase during a day's run which became more pronounced after 10 hr of operation; they considered stoppages for plant cleaning desirable every 7 to 9 hr.

General experience indicates that over rather extended periods plate counts may be relatively high on the products of certain plants and that corrective measures in the operation of the plants reduce the counts materially, without any change in the quality of the original milk. Plate counts on a series of samples taken during a run of powder can be used to detect excessive numbers of organisms from any source, such as the original milk, growth during holding of the milk or the concentrate from it, excessive contamination from equipment, etc. Microscopic counts on the finished powder can be used to determine whether there ever were excessive numbers of bacteria in the material.

Effect of Drying on Various Organisms. In considering the effect of drying on organisms, it is necessary to distinguish between actual drying and exposure to heat preparatory to the drying. With spray drying the rapid loss of moisture during the actual desiccation apparently keeps the temperatures low enough to permit survival of some of the organisms that are relatively susceptible to destruction by heat.

The percentage of the cells that survive may be small and, presumably, they are protected in some way that does not apply to comparable cells which are destroyed, although there is a possibility they have a slightly greater heat resistance. With spray drying, adequate heat treatment of the material to be dried favors low plate counts on the finished product.

Cultures of various organisms rather easily killed by heat have been spray dried in milk with good survival. The living cells in the dried culture often die rapidly, although storage in inert gas at relatively low temperatures aids in delaying their inactivation.

Using an experimental roller drier identical with the factory machines except that the length of the rolls was reduced, Hunwicke and Jephcott [23] studied the effect of the process on various organisms. They found nonspore-forming bacteria were completely destroyed whereas bacterial spores were not destroyed in all cases, the number of survivors apparently being dependent on the extent to which sporulation had occurred. Milk powder prepared from milk highly infected with *M. tuberculosis* was incapable of causing lesions in guinea pigs.

In connection with the destruction of organisms during the drying of milk, various attempts to isolate pathogens from milk powder are of interest, although no definite information is available with reference to the milk used for drying. Shrader *et al.*[24] found that the milk powder supply of Baltimore, as represented by 100 samples from widely scattered sources, was free of *M. tuberculosis*. Many of the samples of milk had very high bacterial counts. In a study of dry milk preparations for infant feeding, Giblin and Pourtales [25] found no hemolytic streptococci of the pathogenic type or *M. tuberculosis* in approximately 200 samples. Average bacterial counts on the various brands ranged from 1,000 to 13,000 per g. Fuller and France [26] examined several samples of milk powder for *M. tuberculosis* to determine their safety for feeding calves. The spray-process samples were dispersed in water and injected into guinea pigs; the roller-process samples were fed to guinea pigs. None of the animals became tuberculous.

Crossley and Johnson [5] noted that spray drying destroyed many bacteria but could not in itself be relied on to destroy pathogens since some nonthermoduric species survived.

Relationship of Organisms to Quality and Keeping Quality. Significant flavor deterioration in the original milk, such as development of acid, affects the flavor of milk powder made from it, but there is

no close correlation between the flavor of the original milk and of the milk reconstituted from the powder. Commonly, the processing has a greater effect on the flavor of the finished powder than the flavor of the original milk. Lea *et al.*[27] reported that improvement in the bacteriological quality of the milk supply and more careful control during its passage through the plant produced some improvement in the keeping properties of powders prepared from milk preheated at a lower temperature but had relatively little effect on the keeping properties of the powder when a higher preheating temperature was used. Also, removal of slime by means of a clarifier did not significantly increase the storage life of the powders. Thiel and Pont[6] observed no consistent difference in either the initial flavors or keeping properties of milk powders that could be correlated with the bacteriological condition but noted that the possibility of biological factors in the deterioration of milk powders cannot be excluded.

Keeping Qualities of Reconstituted Milk. The wide variations in numbers and types of organisms in milk powder, and also in the water used for reconstitution, suggest differences in the keeping qualities of the reconstituted products; in some cases organisms in the water probably have a more marked effect on keeping quality than those in the powder. Bacteria added to the product from utensils and equipment used for reconstitution also may be a factor in the spoilage. Higginbottom[16] found no relationship between the keeping quality and the plate count of reconstituted milk powder. Milk properly reconstituted from bacteriologically satisfactory milk powder and water keeps very well at temperatures commonly used for holding milk. When bacterial changes occur in the milk, they frequently result in very objectionable odors. At relatively high holding temperatures, such as 70 to 80 F, objectionable odors may develop rather quickly. With the normal absence of the common lactic acid organisms in the product, growth of organisms producing undesirable changes may be very rapid.

Nichols[18] found that over 90% of the samples of reconstituted milk held at 60 F remained sweet from 2.5 to 3.5 days. Tests made on the samples after 24 hr indicated the flora of the reconstituted milk did not increase as rapidly as that of raw milk. At 98.6 F the predominating flora of the reconstituted milk rendered it acid and clotted it.

REFERENCES

1. *Standard Methods for the Examination of Dairy Products* (Last ed.). American Public Health Association, Inc., New York, N. Y.
2. *J. Dairy Sci.*, **15**, 391 (1932).
3. *J. Dairy Research*, **14**, 175 (1945).
4. *J. Dairy Research*, **14**, 184 (1945).
5. *J. Dairy Research*, **13**, 5 (1942).
6. *J. Council Sci. Ind. Research*, **18**, 373 (1945).
7. *J. Bacteriol.*, **52**, 711 (1946).
8. *Food Research*, **14**, 165 (1949).
9. *Sci. Agr.*, **32**, 19 (1952).
10. *J. Dairy Sci.*, **5**, 570 (1922).
11. *Proc. World's Dairy Congr.*, **2**, 1265 (1923).
12. *J. Assoc. Offic. Agr. Chemists*, **28**, 424 (1945).
13. *Food Research*, **3**, 421 (1938).
14. Downs, P. A. *Bacterial Flora of Milk Powder.* Thesis. 1920. Cornell University, Ithaca, N. Y.
15. *J. Dairy Sci.*, **11**, 516 (1928).
16. *J. Dairy Research*, **13**, 308 (1944).
17. *J. Dairy Sci.*, **5**, 216 (1922).
18. *J. Dairy Research*, **10**, 202 (1939).
19. *J. Dairy Research*, **14**, 135 (1945).
20. *J. Dairy Research*, **13**, 324 (1944).
21. *J. Dairy Research*, **20**, 65 (1953).
22. *J. Dairy Research*, **14**, 378 (1946).
23. *J. Dairy Sci.*, **8**, 206 (1925).
24. *Am. J. Hyg.*, **8**, 386 (1928).
25. *Am. J. Diseases Children*, **41**, 1100 (1931).
26. *Mass. Agr. Expt. Sta. Bull.* 293 (1933).
27. *J. Dairy Research*, **14**, 122 (1945).

14 Freezing Milk and Cream

The freezing of milk has been considered in connection with supplying areas distant from production and also as a convenient means of providing the product in dairy sections. Cream sometimes is frozen during periods of surplus for use when it is in short supply.

Effect on Bacteria. In frozen milk or cream, bacteria do not multiply. The methods by which bacteria obtain their nutrients preclude growth as long as the milk or cream is frozen throughout. Usually, there is a decrease in numbers, the rate being dependent on the resistance of the contained organisms to the unfavorable conditions.

Hilliard and Davis [1] concluded that, with freezing temperatures, there is much less destruction of bacteria in milk or cream than in pure tap water, owing no doubt to physical protection of the bacteria by the colloidal and solid matter in suspension. The degree of cold below freezing was not important in the destruction of bacteria, but crystallization, probably resulting in mechanical crushing, appeared to be. The relationship of mechanical protection from sheering ice crystals given by solutes to viability of bacteria at low temperatures also was pointed out by Sedgwick *et al.*[2]

Weiser and Osterud [3] studied the death of bacteria at low temperatures, using *E. coli* and a suspending medium of 1% peptone or a peptone buffer mixture at *p*H 7.0. They concluded that death by

freezing involves (1) rapid-acting or immediate death caused by freezing and thawing and (2) storage death which is a direct function of time and temperature. Mortality due to immediate death by freezing was marked but did not vary with intensity of the temperature; death occurred at a brief stage in the freezing process during which extracellular ice formation was being completed. Storage death at the higher freezing temperatures was very rapid and was much greater above −22 F than at −22 F or below; at −319 F storage death either did not take place or was not detected in the period (10 hr) studied. Repeated freezing was more lethal than single freezing or storage in the frozen state for a similar period. Freezing was much more lethal than supercooling.

Homogenized Milk. Frozen homogenized milk was developed during World War II to supply fresh milk to patients on hospital ships. Babcock *et al.*[4] found that homogenized milk frozen and stored at 14 F had a slightly flat flavor when thawed after 21 days; after 89 days the flavor was definitely flat and slight separation had occurred. When frozen and stored at −27 F, homogenized milk remained normal 115 days and there were indications it would remain normal for a longer period. Also, there were indications that with freezing and storage at still lower temperatures, for example −40 F, the milk would remain normal longer than when frozen and stored at −27 F. Homogenized milk frozen and stored at one temperature was moved to a lower temperature without causing abnormalities to develop, but when moved to a higher temperature it became abnormal in appearance and flavor.

Trout[5] noted that the effect of freezing homogenized milk solidly, or partially, and then defrosting it differs from the effect of such treatment on unhomogenized milk. The latter generally becomes flaky and oils off as a result of the freezing whereas such changes are less, or absent, in homogenized milk. The homogenized product freezes slightly faster and more homogenously and, upon melting, the casein-laden fat tends to settle out leaving behind a smooth, milky-watery layer, almost transparent, at the surface.

With both homogenized and unhomogenized milk, Babcock *et al.*[6] noted that during freezing at −4 F there was a gradual but constant increase in percentages of protein, total solids, and ash in the unfrozen portions. As freezing progressed, the bottom sections of both types of milk showed a final increase in fat content. The fat content of the homogenized milk increased in the middle section whereas that of the unhomogenized milk decreased slightly. The top section

of the homogenized milk showed slight variations in fat and that of the unhomogenized milk showed a constant increase.

Special Processing Procedures. Attempts to improve the quality of frozen homogenized milk on thawing for use have involved primarily limiting the destabilization of the casein and controlling the development of an oxidized flavor. Precipitation of the casein is limited by (1) use of fine quality milk, (2) addition of sodium citrate before freezing to act as a stabilizer,[7,8] and (3) suitable storage temperatures. Bell and Mucha[9] reported that the temperature range for storing frozen milk and its concentrates (up to approximately 45% solids) without early development of flakiness begins below −8 F and extends to below −17 F. Their data indicated storage should be lower than −10 F but need not be lower than −20 F. The off flavor in the frozen milk is limited (1) by avoiding copper contamination, (2) addition of ascorbic acid,[7,8,10,11] and (3) by special heat treatment during the preparation. In addition to sodium citrate and ascorbic acid, the effect of various materials on both the casein stability and flavor of frozen milk after thawing have been investigated.[7,8]

Babcock *et al.*[12] noted that homogenized milk should be frozen as soon as possible after processing, but if the milk is of good quality it may be kept as long as 120 hr at 35 F before freezing without adversely affecting the keeping quality of the frozen product.

Keeping Quality of Thawed Product. When frozen homogenized milk is thawed, bacterial growth readily occurs, with the organisms present and the temperature of holding determining the rate of multiplication and type of change occurring. Babcock *et al.*[13] reported that the changes in flavor of homogenized milk which had been held frozen and then thawed were similar to those of fresh homogenized milk. As determined by plate counts there was no significant difference between the two.

Concentrated Milk. Concentrated milk (commonly 3 to 1), to be diluted with water for regular use, has been investigated both as a fresh and frozen product. The decrease in volume of the product lowers costs covering refrigeration, containers, transportation, etc., but processing costs are increased. Attempts to move the operation to areas of cheap milk are opposed by many milk processors and regulatory officials who believe that the milk should be of the same sanitary quality as regular fluid milk. Recombined concentrated milk, whether frozen or unfrozen, has a rather flat flavor compared to fresh milk, probably as a result of the vacuum treatment during condensing.[14] The water used for dilution sometimes is a factor in the flavor and/or

keeping quality of the recombined product; also, it should be well cooled to avoid a warm recombined product.

Fresh. Fresh concentrated milk has been marketed experimentally and apparently accepted by consumers when the price was lower than that of an equivalent amount of fresh milk. The product presents no special bacteriological problem. When held in a refrigerator, in either the concentrated or diluted form, various organisms can grow in it and bring about changes, just as they do in pasteurized milk (see p. 221), but with reasonable holding periods difficulties are not encountered with a properly prepared product.

Burgwald *et al.*[15] noted that concentrated milk had excellent keeping quality when fresh and also after 10 days at 34 to 44 F. Concentrate recombined with tap water had poorer keeping quality than pasteurized milk prepared from the same lot of raw milk; this was attributed to psychrophiles in the water. Postpasteurization contamination was found to be a factor in keeping quality. Olson *et al.*[16] reported that bacterial growth took place at an appreciably greater rate in recombined milk than in the concentrated milk from which it was prepared and that bacterial growth was associated with flavor deterioration. In studies designed to indicate what might be expected in the keeping quality of concentrate prepared under ideal sanitary conditions with proper pasteurization, approximately 2, 3, and 5 weeks of satisfactory keeping quality were obtained with storage at 44.6, 39.2, and 33.8 F, respectively.

Frozen. In general, frozen concentrated milk presents the same problems as frozen homogenized milk. Instability of the casein in the product is increased by the concentration of milk solids. Doan [14] noted that fluid milk has a frozen storage life several times that of 3 to 1 concentrated milk. High heat treatments (above 170 F for 15 min) tend to decrease the stability of the casein on freezing. When holding is to be continued for some time, low storage temperatures should be used. Frozen concentrated milk is less subject to rapid oxidation than frozen normal milk because both homogenization and concentration have antioxidative effects.[14] Proper heat treatment and addition of an antioxidant aid in limiting development of an oxidized flavor.

Doan and Leeder [17] noted that during frozen storage the bacteria in concentrated milk remained dormant and some were destroyed; after 10 weeks the count was almost invariably lower than immediately after freezing.

Concentrated Skim Milk. Skim milk is concentrated for use in various products and is held both unfrozen and frozen. The unfrozen material sometimes is held poorly refrigerated or for excessive periods, with the result that bacteria multiply in it and even cause conspicuous off flavors. In the frozen material instability of the casein is largely corrected by homogenization of the product in which it is to be used.

Cream. Freezing pasteurized cream of relatively high fat content provides a convenient means of storing milk fat. Frozen cream has been used largely in ice cream but also in other products. Excess supplies for which no more profitable market could be found have been churned. Physical changes during freezing may present some difficulties in its use but ordinarily homogenization, or a comparable procedure, largely overcomes these. Trout and Scheid [18] reported that the maximum destabilizing effect in the freezing and storage of cream occurred within the first 3 months. Bell and Sanders [19] noted that differences in milk, especially in size and number of fat globules, and changes in the temperature of the milk and cream greatly influence stability of the cream emulsion, as measured by the amount of oiling off after freezing and brief storage. Later,[20] they concluded that the following precautions give frozen cream which will have a stable emulsion and good body when thawed after storage for several months: (1) Use of freshly drawn milk which contains small, unclumped fat globules, (2) maintenance of these globules in a fluid, completely dispersed state until freezing, (3) quick freezing to maintain this dispersion, and (4) storage temperatures sufficiently low to prevent physical changes which cause localized crowding, distortion, and disruption of the fat globules and consequent oiling off on thawing.

Fabian and Trout [21] found that the plate count of bacteria in cream decreased during storage at −5 to −10 F. With raw cream the averages of 12 monthly samples were 2,498,000 per ml for the fresh cream, 1,638,000 after 6 months, and 680,000 after 12 months. Corresponding values for cream pasteurized at 150 F for 30 min were 6,795, 6,681, and 5,458, respectively. They concluded that from a bacteriological standpoint there is no reason why clean, wholesome, fresh cream cannot be pasteurized and stored for 1 yr in glass, paper, or tin containers at −5 to −10 F.

Off flavors may develop in frozen cream, especially an oxidized flavor. Dahle [22] concluded that pasteurization of the cream at temperatures above 170 F, low copper content, low storage temperatures (0 to −15 F) in proper containers, and homogenization at the heating temperature all aid in enhancing keeping quality. According to

Trout and Scheid.[23] fresh high-quality cream pasteurized at 165 F for 15 min, or at 185 F for 5 min, withstood storage at −10 F for 3, 6, or 12 months without appreciable development of oxidized flavor; 185 F for 5 min resulted in slightly superior keeping quality, particularly after 24 months. With 1 ppm copper added to the cream after pasteurization, the cream invariably became oxidized, even after 3 months, regardless of the temperature of pasteurization. Antioxidants have been used to limit the development of an oxidized flavor in frozen cream.[24, 25, 26]

The defrosting of cream presents a definite bacteriological problem. Because of the large containers commonly used for it, the frozen cream may take considerable time to thaw, with opportunities for bacterial growth in the melted portions unless the process is controlled.

Unintentional Freezing of Milk and Cream. In certain areas during cold seasons, poorly protected milk and cream may be partially or completely frozen on farms or on their way to processing plants. The freezing controls the growth of bacteria in the frozen portions, but the stability of the products is influenced by extent of the freezing, acidity, etc.

REFERENCES

1. *J. Bacteriol.*, **3**, 423 (1918).
2. *Abstr. Bacteriol.*, **1**, 49 (1917).
3. *J. Bacteriol.*, **50**, 413 (1945).
4. *J. Dairy Sci.*, **29**, 699 (1946).
5. Trout, G. M. *Homogenized Milk*, 137, 1950. Michigan State College Press, East Lansing, Mich.
6. *J. Dairy Sci.*, **37**, 1416 (1954).
7. *J. Milk and Food Technol.*, **12**, 101 (1949).
8. *J. Dairy Sci.*, **32**, 175 (1949).
9. *J. Dairy Sci.*, **35**, 1 (1952).
10. *J. Dairy Sci.*, **31**, 951 (1948).
11. *Food Technol.*, **4**, 297 (1950).
12. *J. Dairy Sci.*, **31**, 811 (1948).
13. *J. Dairy Sci.*, **31**, 805 (1948).
14. *Food Technol.*, **6**, 402 (1952).
15. *Milk Dealer*, **42** (2), 50 (1952).
16. *J. Dairy Sci.*, **36**, 817 (1953).
17. *Food Inds.*, **16**, 7, 76 (1944).
18. *J. Dairy Sci.*, **26**, 619 (1943).
19. *J. Dairy Sci.*, **28**, 581 (1945).
20. *J. Dairy Sci.*, **29**, 213 (1946).
21. *J. Dairy Sci.*, **26**, 959 (1943).
22. *J. Dairy Sci.*, **24**, 245 (1941).
23. *J. Dairy Sci.*, **26**, 609 (1943).
24. *J. Dairy Sci.*, **31**, 1024 (1948).
25. *J. Dairy Sci.*, **32**, 301 (1949).
26. *J. Dairy Sci.*, **35**, 93 (1952).

15 Bacteriology of Ice Cream

Bacterial counts are employed in controlling methods of producing and handling ice cream in the same way they are used in supervising milk supplies. Although most of the bacteria in it, as in milk, are harmless, the general conditions which permit the presence of large numbers of organisms are those most likely to result in inclusion of harmful types. Bacterial counts, in combination with suitable inspection of the plant and the methods of production, make possible the adequate sanitary control of ice cream.

The bacteriological principles involved in the manufacture of ice cream are essentially the same as those involved in the handling of market milk, since bacteria are undesirable in both products and should be kept out as completely as possible. However, the variety of materials used in ice cream and the conditions of manufacture and holding present problems not encountered with market milk.

Determination of Numbers of Bacteria. Both the plate method and the microscopic count are employed in determining numbers of bacteria in ice cream, the plate method being used more extensively. The detailed procedures have been adapted from those devised for the examination of milk and have been standardized.[1] Sampling methods for ice cream and procedures for examination of the ingredients also have been developed.

Plate Method. With the plate method, the portion of ice cream to be examined is either weighed or measured. Fay [2] compared the gravimetric and volumetric methods, using 10-g, 10-ml, and 1-ml portions. He concluded that the errors introduced by the various sampling procedures are overshadowed by the error of the plating method and that it makes little difference which of the portions is used. A 10-g portion was suggested for the occasional sample from which air bubbles cannot be eliminated.

Various media are employed in plating ice cream, the medium for milk analysis being used more extensively than any other. Fay [3] reported that in making counts on ice cream with nutrient agar large numbers of pin-point colonies were noted in low dilutions (1 to 100), whereas in higher dilutions (1 to 1,000) these were not in proportionate numbers or were absent. On replating the samples on sucrose agar, the pin-point colonies were present in all dilutions and in proportionate numbers; essentially the same results were obtained by using nutrient agar and adding sterilized ice cream to the higher dilution plates. Fabricius and Hammer [4] noted that when nutrient agar was used in plating ice cream and ice cream mix, occasional samples were encountered with which the different dilutions failed to show a reasonable agreement. Serious discrepancies usually involved the 1 to 1,000 and 1 to 10,000 dilutions. Addition of 1% sucrose to the medium eliminated the discrepancies and also resulted in an increase in size of the colonies.

The temperature at which the plates are incubated greatly influences the results obtained on ice cream. Ayers and Johnson [5] found that 86 F (30 C) for 5 days gave practically double the count obtained at 98.6 F (37 C) for 48 hr. Ellenberger [6] noted that when the plates were incubated 5 days at 86 F the average count of the samples examined was almost 2.5 times the count obtained with an incubation of 48 hr at 98.6 F; with an incubation of 7 days at 68 F (20 C) the count was more than 3 times as high. Pederson and Yale,[7] using an incubation of 48 hr, obtained higher counts at 89.6 F (32 C) than at 98.6 F. Incubation of plates at 89.6 or 95 F (35 C) for 48 hr is the common procedure.[1] In plating ice cream it is essential that standard methods be followed in detail so that various counts can be compared.

Microscopic Count. Fabian [8] adapted the microscopic count to the examination of ice cream. The procedure followed is essentially the same as that for milk. A modification of the microscopic count, intended especially for ice cream and other viscous dairy products, was described by Fay.[9] One-tenth ml of the sample is placed on a clean

slide of the usual size (1 × 3 in.). Two to 4 drops of water (sterile or boiled) are added and the mixture spread evenly over the entire slide. After drying, the smear is treated in the usual manner. When the diameter of the microscopic field is adjusted to 0.157 mm, the bacterial count can be determined by multiplying the average number of cells per field by 1,000,000.

Figure 30. Agar slopes (Burri technic) from interior and surface of dipped ice cream. Colonies indicate heavy contamination of surface of ice cream.

The microscopic count is not used extensively for ice cream. However, the method is useful in detecting the presence of bacteria that fail to grow on plates under certain conditions.

Burri Technic. Long and Hammer [10] applied the Burri smear culture technic to ice cream. Small portions are picked with a sterile needle under a low-power binocular and each is spread over the surface of a dry, agar slope. The portions are intended to weigh 0.02 mg and are picked from firm ice cream, the product being surrounded with ice if necessary. The binocular aids in keeping the portions fairly uniform in size and in actually picking each portion rather then scraping it from a relatively large area. Ordinarily, 10 portions are picked from a sample but larger or smaller numbers may be used for more

or less detailed results. There is considerable variation in the size of the portions picked. Counts with the Burri technic tend to be higher than plate counts. The original surface of the ice cream can be used, a fresh surface can be exposed with a sterile instrument, or the surface and interior can be compared. The method probably has its greatest value in studying the distribution of bacteria in ice cream, particularly in studying the numbers at the surface of dipped ice cream.

Frost Little-plate Method. Bryan *et al.*[11] found with ice cream that incubation of Frost little plates at 98.6 F (37 C) in a moist chamber for a minimum of 8 hr gave counts comparable with standard plate counts. In ice cream containing seeds, nuts, or chocolate, particles may confuse the technician unless the product is diluted 1 to 10 or 1 to 100 before making the little plates.

Coliform Counts. Ice cream frequently is examined for coliform organisms, using essentially the methods developed for milk.[1] The organisms often are found in ice cream with very low bacterial counts; the strains may be relatively heat resistant, but often they are not and represent contamination from equipment, personnel, flies, etc.

Fournelle and Macy [12] found the range of coliform counts, in terms of most probable numbers, on 69 factory-packed samples of ice cream and sherbet was from 0 to 9,180 per ml; with 30 scoop samples of ice cream the range was from 0 to 101,000. They concluded that ice cream should contain <10 coliform organisms per ml. Other investigations [13] indicate that a coliform standard might be established for ice cream in the region of 10 per ml with a provision that 3 out of every 4 samples comply.

Recent analyses on 246 samples of factory-packaged ice cream ($\frac{1}{2}$ gal) at Purdue University showed that 162 (65.9%) of the samples contained <10 coliform bacteria per g, 30 (12.2%) contained 10 to 49, 21 (8.5%) contained 50 to 99, 19 (7.7%) contained 100 to 499, 4 (1.6%) contained 500 to 999, and 10 (4.1%) contained $>1{,}000$. There was no general relationship between coliform and total counts; samples with high total counts had both high and low coliform counts, and coliforms sometimes accounted for an appreciable percentage of the total count of low-count samples. Usually, samples with high total counts and low coliform counts contained primarily heat-resistant bacteria.

Coliform counts on ice cream must be interpreted with caution because of the possibility of false positive presumptive tests. Ice cream often contains one or more fermentable carbohydrates other

than lactose, and acid production in solid media, or gas production in liquid media, may be due to fermentation of carbohydrate in the inoculating material rather than in the medium. Also, with certain types of fruit ice cream, the fruit may contribute sufficient carbohydrate to influence the test. Because of the possibility of false positive presumptive tests, confirmatory tests should be made.

Reduction Tests. The resazurin [14] and methylene blue tests [14, 15, 16] have been used for determining the general bacteriological quality of ice cream. The tests are not used extensively for the same reason that they are not used with pasteurized milk.

Numbers of Bacteria in Ice Cream. Bacterial counts on ice cream vary widely, owing primarily to differences in quality of the materials used and in methods of manufacture and handling. With well-controlled production, plate counts may be as low as a few thousand, or even <1,000, per ml; with careless methods they may be in the millions.

Total counts made recently at Purdue University on 246 samples of factory-packaged ice cream (½ gal) showed that 109 (44.3%) of the samples had plate counts of <5,000 per g, 66 (26.8%) were in the range of 5,000 to 24,999, 26 (10.6%) in the range of 25,000 to 49,999, 16 (6.5%) in the range of 50,000 to 99,999, 16 (6.5%) in the range of 100,000 to 499,999, and 13 (5.3%) had counts of >500,000.

During recent years there has been a significant decrease in plate counts on ice cream as a result of the attention given the sanitary quality of the product by manufacturers and control officials. Quality of the ingredients has improved, more efficient pasteurization has been developed, and contamination of the product from various sources has been reduced. The product of a plant occasionally shows a significant increase in count for no apparent reason. However, study of the general operation, with the examination of samples taken at various points, usually indicates the cause of the increase; poorly cleaned equipment, inadequate heat treatment, high-count ingredients added after the pasteurization, etc., may be involved.

Types of Bacteria in Ice Cream. Studies on ice cream have resulted in the isolation of a wide variety of bacterial species, including micrococci, streptococci, aerobic and anaerobic spore-formers, *Escherichia-Aerobacter* types, *Pseudomonas* species, etc. Action on sterile milk of the organisms in ice cream varies widely. Acid-forming species, both coagulating and noncoagulating, sometimes make up a considerable percentage of the flora. The small size of the colonies frequently present on plates may be due to the species involved, the incubation

temperature, or other causes.[4,6,17] From the standpoint of the general types of organisms present, ice cream is comparable to pasteurized milk or cream. If ice cream is held at a temperature favorable for growth, various bacterial species develop and produce conspicuous changes; yeasts and molds also may multiply.

SOURCES OF BACTERIA IN ICE CREAM

The bacteria in ice cream come from two main sources, (1) ingredients used, and (2) contamination incident to manufacture and handling.

Bacteria in Ingredients Used

The ingredients that require consideration from the standpoint of the bacteria in ice cream are (1) cream, (2) other milk derivatives, (3) stabilizers, (4) sugar, (5) flavoring materials, and (6) coloring materials.

Cream. The cream employed in ice cream varies widely in bacteriological condition. It may be skimmed at the plant from milk of excellent quality, or come from milk of poorer quality and be shipped, inadequately refrigerated, for long distances. In general, during periods of heavy demand for cream the ice cream manufacturer may be less critical than during periods of surplus. Pasteurization of the cream and/or ice cream mix normally destroys a high percentage of the bacteria originally in the cream. Both the numbers and percentages of organisms destroyed depend on the species present, exposures used, etc., but regularly are high with satisfactory methods. Pasteurization of the cream and low holding temperatures, such as 32 F, make it possible to hold cream for reasonable periods. Cream reconstituted from frozen cream, butter, butteroil, etc., using fresh milk or milk powder, presents the same general problem as fresh cream.

Other Milk Derivatives. The milk derivatives other than cream that are employed in ice cream mix include especially products intended to raise the content of solids not fat, although some of them also influence the fat content. Each may be a source of many organisms, but some contain large numbers more often than others. With adequate control of the original quality and proper handling, relatively small numbers of bacteria come from them.

Stabilizers. In general, stabilizers are comparatively unimportant sources of bacteria in ice cream, particularly since they are used in such small percentages.

At one time gelatin from certain suppliers contained large numbers of bacteria while other lots contained only small numbers. Ellenberger [6] reported counts ranging from 430 to 2,396,000 per g. The bacteria present are not especially heat resistant.[6, 18, 19] At present, most samples of gelatin contain relatively few bacteria. Other stabilizers, or stabilizer-emulsifier combinations, contain few bacteria. The important point from the bacteriological standpoint is to protect the products from dust, or other contamination, during storage and use.

Sugar. In general, sugar contains only small numbers of bacteria and is of little importance as a source of bacteria in ice cream. Ellenberger [6] obtained counts that varied from 20 to 255 per g; those of Fay and Olson [20] ranged from 20 to 240. Requirements of canners, soft-drink manufacturers, etc., have emphasized the importance of organisms in sugar and resulted in microbiological control of the product by manufacturers. Exposure of sugar to dust and dirt in plants results in an increase in the bacterial content.

The tentative standards [21] for micro-organisms in bottlers sugar (dry granulated) are: The sugar shall be packed in multiwall paper bags, or equivalent sanitary packages or bulk containers, and shall contain not more than 200 mesophilic bacteria, 10 yeasts, or 10 molds per 10 g. Owen [22] found that paper bags used for packaging sugar are a significant source of yeasts and molds. The outside surfaces of bags sometimes collect considerable extraneous matter and micro-organisms which may be added to ice cream mix during emptying. To avoid such contamination, some plants remove the first layer of paper from the bag before emptying so as to present a clean surface.

Liquid sugar is used in certain plants for manufacture of ice cream. The high solids content prevents growth of many micro-organisms, but there are possibilities of contamination from it. In some plants the liquid sugar is held warm so that it will flow freely and such a procedure has resulted in growth of thermophiles. Condensation at the surface of the sugar may lower the solids sufficiently so that micro-organisms can grow there.

Flavoring Materials. Flavoring materials should be recognized as a source of organisms in ice cream. The early studies of Newman and Reynolds [23] showed the great variations in the numbers of organisms in these materials. With 5 samples of vanilla extract Hammer [24] found counts from 200 to 2,300 per ml. Prucha [25] examined 13 samples from commercial ice cream plants; yeasts and mold counts ranged from 0 to 20 per ml and bacterial counts from 30 to 60,000.

The bacteriological condition of nut meats has been studied extensively because of the conditions under which some of them are prepared. Counts as high as 470 yeasts and molds and 1 million bacteria per g, or as low as 0 per g, have been reported.[25] In general, nut meats in closed containers are the most satisfactory,[26, 27] especially if treated prior to packaging. Aerobic spore formers are important contaminants of nut meats, also micrococci and *E. coli*.[27]

Ostrolenk and Hunter [28] found that nuts in unbroken shells do not contain coliform organisms. They examined nut meats, representing 11 varieties, for *E. coli*. With 200 retail market samples, the organism was isolated from 8 of the 10 varieties of nuts in 0.01-g portions, the percentages ranging from 4 to 45; with 234 samples from 38 commercial shelling plants, all but one of the varieties showed the organism in from 6 to 68% of the samples; with 114 imported samples, all but one of the varieties showed the organism in from 14 to 62%. Artificially contaminated nut meats stored at room temperature contained viable *E. coli* for approximately 68 days. When large numbers of *E. coli* were present on nut meats, correspondingly large numbers of *A. aerogenes* and intermediates were found.

Erickson and Colmer [29] noted that heating black walnut meats in a kiln at 150 F for 30 min with 80% relative humidity did not adequately reduce the numbers of *E. coli;* with 90% relative humidity the percentage of organisms destroyed was increased but the quality of the meats was impaired. Heating at 160 F for 30 min with 70% relative humidity gave satisfactory destruction of *E. coli* when the moisture content of the meats was 12 to 21%. With nut meats of lower moisture content, 180 F for 30 min with 70% relative humidity was satisfactory. Use of 200 F for 15 min with 50% relative humidity gave good destruction but the nuts had a milder flavor and were brittle.

Tracy [30] concluded that nut meats should be dipped in boiling sugar solution (about 50%), to which 1% salt has been added, for 15 sec and dried in a 482 F oven for 2.5 min; treated nut meats should be stored in glassine bags or tin cans at room temperature.

Prucha [25] noted that 13 samples of frozen-pack strawberries contained from 0 to 19,000 yeasts and molds per g and from 10 to 67,000 bacteria. Nine samples of maraschino cherries had from 0 to 110 yeasts and molds and from 0 to 1,780,000 bacteria. Tracy [30] found 8 samples of fresh strawberries contained from 300 to 839,000 bacteria per g. He reported that fruits with delicate flavors, such as fresh strawberries and raspberries, can be pasteurized satisfactorily by mixing with sugar and heating at 145 F for 30 min; if necessary, certain

flavor extracts can be heated at 145 F for 30 min without serious injury to the flavor.

Mundt *et al.*[31] found that coliform bacteria generally are present on, or in, strawberries and are more prevalent with dirty and unsound fruit. Of 78 samples examined, the most probable numbers of coliforms ranged from 0 to >1,600 per g. Some reduction in the number of positive presumptive tests was obtained by washing berries with water or chlorinated water (6 ppm). Confirmation of presumptive positive tests amounted to 37.5 to 50%, depending on the media employed. The organisms responsible for false positive presumptive tests were slow lactose fermenters (*Paracolobactrum intermedium*) which produced gas by fermentation of the sugar contained in the inoculum.

According to De Pew,[32] coliform organisms are most prevalent in banana ice cream, followed by peach, strawberry, vanilla, and chocolate ice cream, in decreasing order. Barber [33] noted that false positive coliform counts were associated with banana, peach, and strawberry ice cream. Gram-negative short rods, gram-positive cocci, and a few yeasts, all of which fermented sucrose, were responsible for the false tests.

Coloring Materials. The possibility of coloring materials adding organisms to ice cream is evident from the following data:

Type of Sample	Number of Samples Examined	Range of Total Bacterial Counts	Investigator
Liquid color from plant		0 to 15,000,000 per ml	Fabian [34]
Powder used at plant	8	0 to 3,300 per g	Smallfield [35]
Powder from supplier	8	0 to 6,500 per g	Smallfield [35]
Liquid color prepared at plant	26	2,700 to 198,000,000 per ml	Smallfield [35]
Liquid color from supplier	9	0 to 13,750,000 per ml	Smallfield [35]
Liquid color from plant	111	0 to 14,000,000 per ml	Tracy [30]

In one investigation [34] 35% of the samples contained coliforms and in another [30] 12.6%. Since color solutions generally are added after pasteurization, contamination from them assumes considerable importance.

Smallfield [35] suggested controlling bacteria in colors by dissolving the dry color in 45% sucrose solution and boiling 2 min or by adding a mixture of dry color and pulverized sugar directly to the mix in the freezer. Tracy [30] advised preparing color solutions with water at 180 F and use of clean containers. Also, he recommended washing and bactericidal treatment of measuring graduates, cold storage of color solutions, and reheating old solutions before use.

Bacteria from Contamination Incident to Manufacture and Handling

Contamination incident to the manufacture and handling of ice cream comes from (1) utensils and equipment, (2) employees, and (3) air.

Utensils and Equipment. In an ice cream plant, cans, vats, coolers, etc., may be sources of organisms, and homogenizers and freezers are especially important in this connection because of their construction.

Hammer and Goss [36] studied contamination from a freezer by adding sterile water and then operating it for about the freezing period, after which the bacterial content of the water was determined; the freezer was being carefully washed after use but not before the next operation. In 5 trials the bacterial counts per milliliter of added water were: 3,700; 141,500; 8,050; 1,195; and 300. The bacterial increase per milliliter of ice cream mix would have been greater than with the water because the latter did not hold air, and larger volumes were required to fill the freezer as full as an ice cream mix would have filled it. Fay [37] reported an instance in which, under plant conditions, the mix before being placed in the freezer contained 26,000 bacteria per g, whereas after freezing it contained 625,000; he considered that part of the increase may have been due to breaking up of clumps of organisms but that most of it probably was the result of a poorly washed freezer.

Dahlberg and Marquardt [38] treated freezers with chlorine solution, hot water, and steam. The chlorine solution did not penetrate the bearings, and bacteria subsequently developed in the wet freezer. The refrigerant in the freezer chilled hot water so that excessive amounts at very high temperatures were required to sterilize. Heating by steam was slow, but a freezer could be sterilized satisfactorily with steam and such treatment was particularly effective as it dried the freezer and sterilized the bearings. A chlorine rinse prior to using a freezer was considered desirable as a supplement to steam sterilization.

Hot water, steam, and chlorine for sanitizing batch ice cream freezers were compared by Fabian *et al.*,[39] the freezers previously having been cleaned by treating with cold water, then with warm water (140 to 150 F) containing washing powder, and finally with hot water (180 F). For sanitizing the front and rear bearings of a freezer, the order of effectiveness was steam, chlorine, and hot water; the rear bearing was harder to sanitize than the front bearing. As judged by the bacterial content of sterile rinse water, chlorine was the best sanitizing agent, steam next best, and hot water least efficient. In general, the preliminary treatment given a freezer was more important than the

method used to sanitize it, since adequate rinsing reduces the bacterial load to a point where sanitizing is greatly facilitated. Shutting off the liquid ammonia during rinsing and sanitizing limits the dissipation of heat.

Employees. The employees in an ice cream plant are a source of organisms in the finished product. Extent of such contamination depends primarily on the personal habits of the individuals involved.

Air. Contamination of dairy products from the air in plants ordinarily is small, although without special air treatment some organisms regularly come from this source. Fabian [40] investigated contamination from the air in an ice cream plant and concluded that it was insignificant. Fewer molds than bacteria were found. Factors increasing the organisms in the air, in what was considered their order of importance, were (1) dry and windy weather, (2) open doors and windows, (3) dry floors, and (4) machinery in operation.

CHANGES IN NUMBERS OF BACTERIA DURING MANUFACTURE OF ICE CREAM

A number of the processes used in the manufacture of ice cream influence the bacterial content. As determined by the plate method, some of them cause decreases but others commonly result in increases because of the breaking up of clumps of organisms.

Making the Mix. Hammer and Sanders [41] noted that an ice cream mix may contain a smaller number of bacteria than the cream used in preparing it. In 8 trials the bacterial content of the mix was 8 to 75% (average 43.6) lower than that of the cream. The smaller number of bacteria in the mix than in the cream may be due, in part, to a volume change, as a result of adding other ingredients to the cream, but extent of the decrease in certain instances is such that other factors must be operative. One of these undoubtedly is the plasmolyzing action of the sugar that makes up from 13 to 18% of the ordinary mix. This plasmolyzing action apparently varies with different species of organisms so that a significant decrease in bacterial count when a mix is made cannot always be expected.

Pasteurizing the Mix. Pasteurization of a mix, either by the LTLT or HTST method, commonly results in the destruction of many bacteria. The exposures used for mix vary widely, but generally they are higher than for market milk because the higher solids content of a mix may afford a protective action to micro-organisms.[42,43]

Dubois and Martin [44] compared the effects of various pasteurization

exposures on the bacterial flora of ice cream mixes, using 145 F for 20, 25, and 30 min, 150 F for 20, 25, and 30 min, 160 F for 10, 15, and 20 min, 170 F for 2, 5, and 10 min, and 180 F flash. In the first trials the pasteurization was carried out in test tubes but, later, quantities of mix were pasteurized under plant conditions. Differences in bacterial destruction by the various exposures were too small to be measurable by the plate method. In mixes made from pasteurized dairy products the flora after pasteurization consisted largely of slow acid formers, together with some peptonizers, alkali formers, and inert types; in mixes made from raw dairy products it consisted of peptonizers, alkali formers, and inert types.

During recent years, there has been considerable interest in HTST pasteurization of ice cream mix because of possible savings in floor space, time, and labor. Dowd and Anderson [45] found that pasteurization at 180 F for 19 sec proved as effective in reducing the number of bacteria in ice cream mix as 160 F for 30 min. The average plate count of the mix prior to pasteurization was 305,000 per ml, with HTST pasteurization after homogenization it was 28, with HTST pasteurization before homogenization it was 36, with LTLT pasteurization after homogenization it was 52, and with LTLT pasteurization before homogenization it was 188. Using a heat-resistant *Micrococcus* as the test organism, Barber [46] showed that exposures of 175 F for 25 sec, or 190 F for 1.4 sec, gave destruction comparable to 155 F for 30 min. Tracy *et al.*[47] noted that mix heated at 175 F for 30 sec showed a pasteurization efficiency comparable to 155 F for 30 min, but it was slightly less favorable than that obtained at 160 F for 30 min. Comparable results were obtained with exposures of 177.5 F for 30 sec and 160 F for 30 min; greater destruction of bacteria occurred with an exposure of 180 F for 30 sec, but the resulting ice cream had a cooked flavor. Additional experiments [48] with a tube heater which had a 6.1 sec coming-up and holding time showed that nonspore-forming test organisms were 99% or more destroyed at 180 F; for comparable destruction of spore formers, temperatures of 240 to 260 F were necessary.

Speck *et al.*[49] found that composition of ice cream mix, within the ranges of 33.4 to 42.4% total solids and 14 to 17% sucrose, had no effect on the destructive efficiency of heat treatments. With a *Micrococcus* as the test organism, exposures of 175 F for 21.2 sec, 180 F for 11.8 sec, 185 F for 7.2 sec, and 190 F for 0.99 sec gave destruction comparable to 155 F for 30 min. With a *Microbacterium* as the test organism, exposures of 175 F for 16.5 sec, 180 F for 11.4 sec, 185 F

for 6.5 sec, and 190 F for 0.27 sec gave destruction comparable to 155 F for 30 min.

Experiments [50] with a Vacreator, operated so that a minimum temperature of 194 F was maintained in the first chamber, indicated that bacterial destruction was equal to, or greater than, that obtained by pasteurization of ice cream mix in a sealed glass tube at 155 F for 30 min. Another investigation [49] indicated that 191.5 F in the first chamber gave an effect equivalent to 155 F for 30 min.

In 1953, the Frozen Desserts Ordinance and Code [51] recommended by the U. S. Public Health Service was amended to provide for a pasteurization exposure of 175 F for 25 sec as an alternative to 155 F for 30 min.

An ice cream mix tends to protect certain organisms during pasteurization. Fabian and Coulter [42] found that ice cream mix has a greater protective action than skim milk for *Escherichia-Aerobacter* organisms when heated at 140 or 145 F for 30 min. None of the ingredients of ice cream alone had such a protective effect. Bartsch [52] reported that a plant having an average logarithmic bacterial count of 800 per g on 166 samples, and using a pasteurization exposure of 165 F for 30 min, frequently had positive coliform tests. It was noted that 4 to 6 in. of foam floated on the surface of the mix at the end of pasteurization. The incidence of coliform bacteria in the foam was 1, or more, per g in 8.55% of the samples and 10, or more, per g in 1.97%.

Fay [43] concluded that addition of sugar to ice cream mix may increase the thermal resistance of the microflora. Hypertonic solutions of dextrose and sucrose made up in broth, milk, water, or ice cream mix afforded certain cells definitely greater protection against heat than when heated in water suspensions. He suggested that certain sugars retard the agglomerating action of heat on the protoplasmic colloids and, as a result, cells subjected to a given heat treatment in concentrated sugar solutions are only reversibly coagulated, whereas in aqueous suspensions the protoplasm more closely approaches the irreversible stage of coagulation. In comparative studies on milk and ice cream, Paley and Isaacs [53] found that the latter exerts a protective action for *E. coli* against a pasteurization temperature of 143 F, the apparent resistance of the organism being approximately doubled. The effect was attributed to stabilizers (locust bean gum and sodium alginate); sugar and gelatin exerted no protective action.

On the basis of the heat resistance of an especially resistant strain of *E. coli*, Myers and Sorensen [54] suggested that a pasteurization tem-

perature of not less than 150 F and preferably 155 F be maintained for 30 min in order to ensure destruction of the coliform group of bacteria in ice cream mix.

There is evidence that the solids in ice cream also protect phosphatase against destruction by exposures that destroy it in milk.[55, 56]

Homogenizing the Mix. Homogenizing a mix usually results in an increase in bacterial content. This may be due, in part, to contamination from the machine, but breaking up of clumps of organisms also is a factor. Fabian and Cromley [57] made counts on mixes after pasteurization and again after homogenization at 2,500 to 3,000 lb pressure. In 74.0% of the 46 comparisons there was an increase ranging from 5.9 to 1,400%, in 10.8% there was a decrease ranging from 9.1 to 86.2%, and in 15.2% there was no change. Peterson and Tracy,[58] in a study of the condensation process of preparing an ice cream mix, noted that in the 2 trials investigated homogenization increased the count.

Aging the Mix. From the data available on storage of milk and cream it would be expected that a properly cooled mix could be aged for a reasonable period without significant increase in organisms. Fay and Olson [59] obtained data indicating that, in general, aging a mix 16 to 24 hr does not markedly affect the bacterial count when the temperature is below 45 F. A mix made by the condensation process was held at 32 to 35 F by Peterson and Tracy; [58] the count originally was 1,400 per ml, after 5 days it was 1,700, and after 14 days 762,000.

The studies of Mueller [60] indicated that a short aging period at a relatively high temperature, followed by aging at a low temperature, increased the basic viscosity and gel strength of both a gelatin ice cream mixture and a pure gelatin water solution, when compared with aging at a low temperature only. Mueller and Frandsen [61] then compared aging ice cream mixes at 68 F and 38 F; the mixes were pasteurized at 145 F for 30 min and homogenized at this temperature, using 2,500 lb pressure. Aging at 68 F for 2, 4, or 6 hr had no measurable effect on the bacterial content. Mueller and France [62] found that aging pasteurized ice cream mixes for 6 hr at 68 F, whether followed by aging for 18 hr at 38 F or not, gave no significant increase in bacterial content; when aged 24 hr at 68 F a definite increase did not occur until about the tenth hour. Aging of mixes for 24 hr at 38 F did not give an increase in bacteria.

Nelson [63] reported that the bacterial count of ice cream mix stored at 40 F, or above, increases with storage and may reach considerable

magnitude as the storage temperature increases. Coliforms usually increased in numbers in ice cream mix at 46.4 F, or above; the increases may lead to false conclusions relative to postpasteurization contamination based upon presence of coliforms in appreciable numbers.

Freezing the Mix. Freezing a mix commonly results in an increase in the bacterial content, even when the condition of the freezer is such that it is not a significant source of organisms. In 51 comparisons of the bacteria in mix and in frozen but unhardened ice cream, Hammer and Goss [36] found that in 43 (84.3%) the count was increased from 2 to 227% (average 46.3), in 6 (11.8%) it was decreased from 2 to 31% (average 13.0), whereas in 2 (4.0%) there was no change. Ellenberger [6] noted an average increase of 48% in the number of bacteria as a result of freezing. Fabian and Cromley [57] made 41 comparisons of the counts before and after freezing; in 46.3% there was an increase, as a result of the freezing, varying from 0.95 to 260% (average 46.4); in 36.6% there was a decrease ranging from 3.85 to 59.7% (average 25.95); and in 17.1% there was no change. In 28 trials Fay and Olson [59] found an average increase in count on freezing; in only 6 of the comparisons was a decrease noted and 4 of these were small enough to be within the limit of experimental error.

The increase in the plate count that so frequently is obtained during freezing an ice cream mix in a clean freezer undoubtedly is due to breaking up of clumps of bacteria during the agitation and, accordingly, should be regarded as an apparent increase. Lowering of the temperature during freezing would be expected to have a destructive influence on certain of the organisms present and this may account for the rather large decreases sometimes noted. The effect of freezing on the bacterial content of a mix is a combination of two distinct and opposing influences, one tending to increase the plate count and the other to decrease it. Presumably, each of these influences has a variable effect, depending especially on the species of organisms present.

Hardening Ice Cream. Hardening ice cream after it comes from the freezer results in a pronounced decrease in its temperature, and this would be expected to destroy some of the contained organisms, extent of destruction being dependent on the species present. Hammer and Goss [36] made 52 comparisons of the bacterial content of unhardened and hardened ice cream; in 45 (86.5%) hardening caused decreases ranging from 2 to 75% (average 39.1), in 6 (11.5%) it caused increases varying from 7 to 22% (average 13.8), whereas in 1 (1.9%)

there was no change. The few increases probably were due to experimental error since there was no opportunity for addition of organisms. In 28 comparisons Fay and Olson [59] found that the average bacterial content before hardening was 236,688 per g and after 2 or 3 days in storage it was 186,320; increases occurred in 8 of the trials which the investigators considered were due to experimental error.

Storage of Ice Cream. Ordinarily, ice cream is stored below 0 F. At such temperatures, growth of micro-organisms would not be expected and investigations [6, 24, 36, 61] have verified this. In most instances the bacterial count shows no change, or it decreases slightly.[24, 36, 57]

Hammer and Goss [36] reported that softening and rehardening ice cream may result in either a significant increase or decrease in the number of bacteria present, the effect presumably being dependent on species of bacteria and extent of softening. An increase seemed to be more common in ice cream that was only slightly softened, whereas a decrease followed pronounced softening, probably because of the more destructive action of the rehardening. Large increases in the bacterial content of ice cream resulting from softening and rehardening are important if a bacterial standard is being enforced.

Manufacture of Ice Cream with Low Bacterial Count. Use of methods that tend to yield ice cream with low bacterial counts is now widespread among well-managed plants. High-quality ingredients are carefully handled in properly sterilized equipment, with just as little opportunity for contamination from human sources as possible, so that a clean and safe product can be marketed. Pasteurizing and homogenizing the entire mix, with the exception of certain ingredients whose bacteriological quality can be maintained satisfactorily, has advantages from a sanitary standpoint. There is relatively little handling after the heating and, accordingly, little opportunity for contamination.

Attempts to produce ice cream with low plate counts often result in values of only a few thousand per milliliter or, occasionally, $<1{,}000$. Such low values are possible only with efficient pasteurization and adequate protection against recontamination. The factor over which the manufacturer has the least control is the quality of the cream and other dairy products and, when these contain many bacteria that are resistant to the pasteurization exposures, counts are relatively high. Unless proper methods are used, cutting and wrapping of bricks and slices, preparation of individual molds, etc., may result in contamination of the product because of the special handling involved.

CONTAMINATION OF ICE CREAM BY VENDORS

Ice cream is consumed extensively in public eating establishments of various types under conditions that necessitate its being dipped from large containers. The dishes used are possible sources of contamination but, because of the way in which dippers and scoops ordinarily are held, they appear to be especially important in this connection.

Krog and Dougherty [65] took samples of the same bulk ice cream with the vendor's scoop, and also with a sterile spoon, from a freshly exposed surface after removal of at least 1 in. of surface ice cream with another sterile spoon. Bacterial counts invariably were higher on the scoop samples, and often the difference was striking. Water in which the scoops were kept showed counts from 4,000 to 6,336,000 per ml. With better care of the scoop, including rinsing in hot or cold running water before and after each use, contamination from the scoops was largely eliminated. A survey by Fabian and Hook [66] of 96 establishments retailing ice cream indicated the total bacterial count, as well as the coliform count, was lower when running water instead of still water was used in dipper receptacles. The speed at which the water was running through the receptacles also was of importance.

Abele [67] found that bacterial counts on water in which ice cream dippers were immersed ranged from 600 to 20,000,000 per ml, with some plates too heavily seeded to count. The count on water said to be changed every other day or so was 6,900,000 per ml; the average (logarithmic) count on 5 samples said to be changed once daily was 2,400,000, that on 4 samples said to be changed twice daily was 79,000, that on water said to be changed 10 times daily was 3,500,000, and that on water said to be changed 15 times daily was 210,000. The average count on water in 5 receptacles into which a small stream of water constantly was flowing was 9,500 per ml (from 600 to 77,000). Blair [68] reported ice cream scoops were being immersed between use in very turbid water, having bacterial counts of 540,000 and 297,000 per ml, respectively.

With the Burri technic, dipper contamination can be studied by comparing numbers of organisms at the interior and surface of dipped portions of ice cream. Surfaces of some of the samples yield strikingly larger numbers of colonies than the interiors.[10]

When ice cream is dipped for consumption elsewhere, the container

may be an additional source of contamination. Speck and Black [69] found the bacterial counts of open-top ice cream containers obtained from retail establishments varied widely; they apparently were correlated with the storage conditions in the stores. Many of the containers yielded more than 1 organism per ml capacity. Contamination from air and dust probably was the most important factor influencing the counts. Typical organisms contaminating the paper containers belonged to the genera *Micrococcus, Alcaligenes, Sarcina, Bacillus,* and *Achromobacter.*

BACTERIAL STANDARDS

Bacterial standards for ice cream have not been established and enforced to the extent of standards for milk. In 1950, Dahlberg and Adams [70] reported that 10 of the states had no sanitary laws regulating the sale and manufacture of ice cream. The predominating bacterial standard established by state laws was 100,000 per g, or ml, but only 19 states had any standard. Pasteurization standards among states varied from 140 F for 25 min to 155 F for 30 min with LTLT pasteurization and from 160 F for 15 sec to 161 F for 16 sec, or 170 to 172 F with no holding time, for HTST pasteurization. The widespread sources of ingredients for making ice cream and the general intercity and interstate shipment of ice cream tended to complicate inspection and control.

The usual standards for ice cream are met easily when good ingredients and suitable manufacturing procedures are employed.

PRODUCTS RELATED TO ICE CREAM

Products which vary from ice cream in the amount of butterfat and other ingredients contained, and also products in which butterfat is replaced with other fats, especially vegetable fat, are consumed in large volumes in some areas. In general, these products present the same bacteriological problems as ice cream and bacterial counts are useful in their supervision. The vegetable fat used in some mixes does not present a bacteriological problem since it is a poor growth medium. The milk or milk products generally used in all mixes influence the bacterial counts of the products and deserve the same considerations as for ice cream. The processing of mixes for products related to ice cream is carried out in the same manner as ice

cream mix, with similar or identical equipment. The bacteriological standards generally conform to those of ice cream.

Ripper *et al.*[71] examined 54 samples of ice milk purchased from retail stores. The plate counts of 46 samples (85.2%) were <50,000, 43 (80%) had direct microscopic counts of 200,000 or less, and 40 (74%) had 10 or less coliforms per ml. Five samples (9.3%) had total plate counts of >100,000, 11 (20.4%) had microscopic counts of >200,000, and 14 (25.9%) had coliform counts of >10 per ml.

The numbers of bacteria in fountain-mixed milk drinks were determined by Fiske.[72] Since no standards were established for mixed milk drinks, the quality standard for grade A milk was adopted by the author. Of 118 samples taken from the mixing container, 50% contained >30,000 bacteria and 42% contained >10 coliforms per ml. When samples were taken from the drinking utensil, 53% contained >30,000 bacteria and 44% contained >10 coliforms per ml. Of the samples of ice cream used in the drinks and obtained with the fountain dipper or scoop, 30% had >50,000 bacteria and 49% had >10 coliforms per g. Tests on 115 samples of chocolate syrup used in milk drinks showed that 34% contained >30,000 bacteria but only 6% contained >10 coliform organisms per g. Of 119 samples of milk used in milk shakes, 29% contained >30,000 bacteria and 29% contained >10 coliform bacteria per ml. An examination of utensils showed that 66% of the mixing containers, 86% of the ice cream dippers, 75% of the mixer arms and blades, and 26% of the drinking utensils contained more than the swab test standards of 100 organisms per utensil; the results indicated that soda fountain utensils may not be properly sanitized.

REFERENCES

1. *Standard Methods for the Examination of Dairy Products.* (Last ed.) American Public Health Association, Inc., New York, N. Y.
2. *J. Dairy Sci.*, **13**, 40 (1930).
3. *J. Bacteriol.*, **11**, 96 (1926).
4. *Ia. Agr. Expt. Sta. Bull.* 285 (1931).
5. *U. S. Dept. Agr. Bull.* 563 (1917).
6. *N. Y. (Cornell) Agr. Expt. Sta. Mem.* 18 (1919).
7. *Am. J. Public Health*, **24**, 477 (1934).
8. *J. Dairy Sci.*, **8**, 246 (1925).
9. *J. Dairy Sci.*, **16**, 311 (1933).
10. *Ia. State Coll. J. Sci.*, **14**, 163 (1940).
11. *J. Dairy Sci.*, **25**, 827 (1942).
12. *J. Dairy Sci.*, **25**, 475 (1942).
13. *Am. J. Public Health Yearbook* 1952–53, **43**, 76 (1953).
14. *J. Dairy Sci.*, **27**, 993 (1944).
15. *Mon. Bull. Minist. Health and Public Health Lab. Service*, **7**, 84 (1948).
16. *Medical Officer*, **79**, 89 (1948).

17. *U. S. Dept. Agr. Bull.* 303 (1915).
18. *J. Dairy Sci.*, **8**, 115 (1925).
19. *J. Bacteriol.*, **14**, 363 (1927).
20. *J. Dairy Sci.*, **8**, 415 (1925).
21. *Food Eng.*, **25** (3), 82 (1953).
22. *Food Eng.*, **25** (1), 57 (1953).
23. *Monthly Bull. Calif. Dept. Agr.*, **19**, 677 (1930).
24. *Ia. Agr. Expt. Sta. Bull.* 134 (1912).
25. *J. Milk Technol.*, **2**, 127 (1939).
26. *Food Research*, **4**, 191 (1939).
27. *Proc. Food Conf. Inst. Food Technol.*, **1**, 295 (1940).
28. *Food Research*, **4**, 453 (1939).
29. *Food Research*, **13**, 417 (1948).
30. *J. Milk Technol.*, **2**, 118 (1939).
31. *J. Milk and Food Technol.*, **17**, 362 (1954).
32. *Ice Cream Rev.*, **37** (10), 69 (1954).
33. *J. Dairy Sci.*, **38**, 233 (1955).
34. *Ice Cream Field*, **16** (6), 16 (1930).
35. *Ice Cream Trade J.*, **29** (3), 41 (1933).
36. *Ia. Agr. Expt. Sta. Bull.* 174 (1917).
37. *J. Dairy Sci.*, **6**, 283 (1923).
38. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 628 (1933).
39. *J. Dairy Sci.*, **25**, 1 (1942).
40. *Mich. Agr. Expt. Sta. Tech. Bull.* 83 (1927).
41. *Ia. Agr. Expt. Sta. Bull.* 186 (1919).
42. *J. Dairy Sci.*, **13**, 273 (1930).
43. *J. Agr. Research*, **48**, 453 (1934).
44. *J. Dairy Sci.*, **16**, 435 (1933).
45. *J. Dairy Sci.*, **26**, 37 (1943).
46. *Ice Cream Trade J.*, **47** (6), 52 (1951).
47. *Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 47th Ann. Conv.*, **2**, 38 (1951).
48. *Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 46th Ann. Conv.*, **2**, 21 (1950).
49. *J. Dairy Sci.*, **37**, 37 (1954).
50. *J. Dairy Sci.*, **33**, 820 (1950).
51. *Frozen Desserts Ordinance and Code Recommended by U. S. Public Health Service*, 1940.
52. *Southern Dairy Products J.*, **54** (1), 51 (1953).
53. *J. Dairy Sci.*, **24**, 421 (1941).
54. *Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 36th Ann. Conv.*, **2**, 7 (1936).
55. *J. Dairy Sci.*, **22**, 219 (1939).
56. *J. Dairy Sci.*, **22**, 261 (1939).
57. *Mich. Agr. Expt. Sta. Tech. Bull.* 60 (1923).
58. *J. Dairy Sci.*, **5**, 273 (1922).
59. *J. Dairy Sci.*, **7**, 330 (1924).
60. *Ind. Eng. Chem.*, **25**, 707 (1933).
61. *Mass. Agr. Expt. Sta. Bull.* 302 (1933).
62. *Mass. Agr. Expt. Sta. Bull.* 314 (1934).
63. *J. Dairy Sci.*, **27**, 459 (1944).
64. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 83 (1915).
65. *Am. J. Public Health*, **27**, 1007 (1937).
66. *Rept. Proc. Intern. Assoc. Ice Cream Mfrs., 36th Ann. Conv.*, **2**, 30 (1936).
67. *Ice Cream Rev.*, **22** (3), 74 (1938).
68. *New Zealand J. Sci. Technol.*, **25**, 260 (1944).
69. *Food Research*, **2**, 567 (1937).
70. *Bull. Natl. Research Council (U. S.)*, 121 (1950).
71. *J. Milk and Food Technol.*, **16**, 121 (1953).
72. *J. Milk and Food Technol.*, **15**, 167 (1952).

16 Bacteriology of Lactic Cultures

Lactic (lactic acid) cultures (formerly often called starters) are used extensively in the dairy industry to produce acid and/or flavor in such products as butter, cultured buttermilk, cultured sour cream, and various cheeses. In general, the acid developed by the cultures also tends to prevent, or limit, growth of objectionable bacteria in the products.

Lactic cultures contain two distinct types of bacteria. One type produces primarily lactic acid from lactose, and the other type ferments citric acid with formation of various compounds, at least some of which are important from the flavor standpoint.

When lactic cultures are used in butter plants, they commonly are referred to as butter cultures. Properly selected and propagated cultures give butter a high flavor that is essential in many markets, particularly markets for unsalted table butter. Cultures tend to mask certain off flavors, such as feed flavors and those absorbed by cream. A culture suitable for flavor development in butter generally is satisfactory for use in the manufacture of cultured buttermilk, cultured sour cream, or cheese.

In the cheese industry, lactic cultures commonly are referred to as cheese cultures. The most important quality of a cheese culture is acid production which is essential during the manufacturing, and the acid-producing organisms also appear important in the ripening of

some cheeses. Fermentation of citric acid is of little significance with a cheese culture and sometimes pure cultures of lactic acid bacteria are employed with satisfactory results.

History of Lactic Cultures. The first use of cultures in butter making was to control the flavor, and early attempts to achieve this result consisted of addition of clean-flavored sour milk, or buttermilk from a churning of fine butter, to cream intended for churning. Cultures of this type are known as natural starters and a common procedure is to transfer a portion of such material to pasteurized milk and permit the organisms to grow. Some satisfactory cultures have been developed by this method, but chances of obtaining a good culture are limited.

Conn [1] was the first bacteriologist in the United States to conduct extensive experiments on the influence of bacteria on cream ripening. He recognized the complexity of the process and doubted whether any one organism would produce the desired result. Also, he pointed out that in cream ripening something besides souring takes place.[2] The acid produced in ripening was considered to be derived from lactose, and the flavor from some other constituent.[3]

According to Knudsen,[4] use of selected bacteria for cream ripening was the result of the work of Storch. He isolated cultures of bacteria from buttermilk, butter, and ripened cream and determined their ability to produce a high flavor in butter. One of the cultures isolated produced an aroma typical of ripened cream. Storch supplied cultures for inoculating the heated cream when the first trials on pasteurization of cream intended for butter were carried out in 1888. Fine-flavored butter was not produced by these cultures, but souring defects were prevented.

Commercial cultures for cream ripening were available in Europe about 1890. Their use increased rapidly in Denmark where, in 1897, 802 of 866 butter plants were using cultures.[5] Conn [6] reported that the commercial cultures studied by him were not all alike. Most were pure cultures, but some were mixtures; 1 culture contained 10 or 12 species.

From the early studies on lactic cultures, it commonly was believed that they contained only lactic acid-producing bacteria. There was little evidence to support this idea because a pure culture capable of producing the desired result had not been isolated. In 1919, three groups of investigators noted the presence of organisms other than the lactic acid bacteria in normal cultures. Hammer and Bailey [7] observed that lactic cultures produce considerable volatile acid in

milk whereas S. *lactis* cultures do not, and they isolated organisms that yielded considerable volatile acid. Storch [8] noted the presence of organisms which he called *x* bacteria in lactic cultures, and Boekhout and Ott de Vries [9] described their "aroma" bacteria.

Since 1919, many workers have found that proper development of flavor in lactic cultures requires the action of two distinct types of bacteria. Recognition of this associative action has emphasized the complexity of the fermentation occurring in cultures. Hammer [10] reported that citric acid is the primary source of the volatile acid formed in lactic cultures by the organisms associated with S. *lactis*. Various observations have confirmed this and also indicated that citric acid is the source of a number of other compounds of importance in flavor development.

In 1929, the studies of van Niel *et al.*[11] and of Schmalfuss and Barthmeyer [12] showed the importance of biacetyl as an aroma constituent of butter and indicated that it is formed through oxidation of acetylmethylcarbinol. These findings stimulated research on the production of acetylmethylcarbinol and biacetyl in lactic cultures. The importance of acetylmethylcarbinol plus biacetyl in lactic cultures was shown by Michaelian *et al.*,[13] who found that cultures having a satisfactory flavor contained comparatively large amounts of these materials, whereas cultures lacking in flavor contained relatively small amounts, or none. Fifty-one cultures having a satisfactory flavor yielded 10 to 39.5 mg nickel dimethylglyoximate (equivalent to acetylmethylcarbinol plus biacetyl per 200 g culture), whereas 41 cultures lacking flavor yielded 0 to 7.4 mg.

Milk cultures of S. *lactis*, or of the organisms associated with it in lactic cultures, ordinarily do not contain significant amounts of acetylmethylcarbinol or biacetyl so that the relatively large amounts of these compounds in lactic cultures are the result of associative action of the two types. Occasionally, a pure culture of a lactic acid-producing *Streptococcus* yields sufficient flavor compounds in milk to affect the flavor [14,15,16,17] but the majority of cultures do not.[13,14,18] Krishnaswamy and Babel [14] isolated an S. *lactis* culture which produced 4.0 ppm biacetyl in milk; this was the largest amount produced by any culture studied. Addition of citric acid to milk cultures of S. *lactis* increased slightly the biacetyl contents, but the increases were not as conspicuous as those obtained by addition of citric acid to lactic cultures. Acetylmethylcarbinol contents of milk cultures of S. *lactis* were not increased appreciably by addition of citric acid, but the volatile acidities were increased considerably.

STREPTOCOCCI ASSOCIATED WITH *S. LACTIS* IN LACTIC CULTURES

General Characters. The organisms normally associated with *S. lactis* in lactic cultures are typical streptococci which are not distinguishable from *S. lactis* microscopically; in pure cultures they often are somewhat smaller than *S. lactis*. The organisms grow poorly on artificial media; tomato, beef infusion, and whey agars are relatively satisfactory for them. Orla-Jensen *et al.*[19] reported the favorable influence of yeast extract, even in small amounts, on the growth and noted that it stimulated acid production from various fermentable materials.

The outstanding character of the organisms is their ability to ferment citric acid. The principal products formed from citric acid are acetic acid, carbon dioxide, acetylmethylcarbinol, biacetyl, and 2,3-butylene glycol. The proportion in which these compounds are produced varies widely with the conditions under which the fermentation occurs, particularly with the *p*H. Biacetyl and acetylmethylcarbinol accumulate in significant amounts only when the *p*H is relatively low.

Nomenclature. The citric acid-fermenting bacteria normally present in lactic cultures have been placed in various genera by different investigators. Hammer[10] named the organisms *Streptococcus citrovorus* and *Streptococcus paracitrovorus*. The fundamental difference between them is that *S. citrovorus* produces no lactic acid in milk, whereas *S. paracitrovorus* forms a variable amount of D(−) lactic acid. Because of this difference *S. citrovorus* does not redden litmus milk, except on extended incubation, and produces a comparatively low volatile acidity; *S. paracitrovorus* reddens litmus milk and produces a comparatively high volatile acidity, the lactic acid tending to make the citric acid more available. The distinction between the two species is not well defined, since the amount of acid produced by *S. paracitrovorus* is so variable. In a study of 124 cultures of *S. paracitrovorus*, Hammer and Baker[20] found the total acidities produced in milk varied from 0.27 to 0.99% (average 0.46), whereas the volatile acidities * ranged from 12.5 to 40.0 (average 28.6) without

* The volatile acid in a culture is determined by steam-distilling a mixture of 250 g of culture and 250 ml of distilled water, after addition of 15 ml *N*/1 sulfuric acid, and titrating the first 1,000 ml of distillate, using *N*/10 sodium hydroxide and phenolphthalein; distillation is at such a rate that approximately 2 hr are required to obtain the 1,000 ml. Results are expressed as the milliliters of *N*/10 sodium hydroxide required. Only a portion of the volatile acid is ob-

added citric acid and from 44.4 to 96.6 (average 73.9) with 0.4% citric acid added to the milk. Some of the cultures coagulated milk; others did not. With an occasional culture, coagulation was rapid.

Knudsen and Sorensen [21] proposed that the organisms be called *Betacoccus cremoris* and described two types, *a* and *b;* type *a* is similar to S. *paracitrovorus.* Hucker and Pederson [22] considered that they belong to the genus *Leuconostoc* and recognized two species, *Leuconostoc citrovorum* and *Leuconostoc dextranicum.*

Various investigators [23, 24, 25] object to the genus name *Leuconostoc* for the organisms since they do not conform to the requirements of this genus. Because of differences in nomenclature, the citric acid-fermenting streptococci characteristic of lactic cultures are conveniently designated the flavor organisms.

Isolation. Isolation of the flavor organisms from lactic culture, or comparable material, ordinarily can be accomplished by plating. The plates are poured with a suitable medium, such as tomato agar, incubated at 70 F (21.1 C), and well-developed colonies picked into litmus milk. Cultures that do not show the typical S. *lactis* reaction are examined for morphology and the general changes produced in milk, including the fermentation products. The comparatively small number of flavor organisms in lactic culture and the uneven distribution complicate their isolation by direct plating. In some cases many colonies include none of these organisms but, occasionally, most of the colonies picked are of this type. Generally, it is easier to isolate flavor organisms from lactic cultures that have been held several days; this suggests that the flavor organisms may be more acid tolerant than S. *lactis.* By inoculating lactic culture on suitable agar slopes and carrying it through a number of transfers, with several days between them, a culture containing only flavor organisms may result.

Benchetrit [26] found that tomato agar modified by addition of a small amount of α-bromopropionic acid is very useful in isolating flavor organisms from lactic culture and other materials since it inhibits S. *lactis.* The acid is added to the melted and cooled agar just before pouring, and the inoculum is either distributed through the medium or smeared on prepared plates. The concentration of acid necessary for inhibition varies with different lots of tomato agar but usually ranges from 0.2 to 0.6 ml of $N/10$ solution per 100 ml of agar. With the correct concentration of acid, growth of S. *lactis* is checked

tained with this procedure so that the method is primarily of value for comparative purposes.

rather effectively without seriously influencing growth of the flavor organisms.

A special medium also has been used for the differentiation and enumeration of *S. citrovorus*.[27]

Distribution. The flavor organisms regularly are present in lactic cultures of satisfactory quality. *S. paracitrovorus* has been isolated repeatedly from sour milk and cream, but *S. citrovorus* is much less common in these materials. Both species sometimes are obtained from butter or cheese, in which case they probably represent organisms derived from the lactic culture used. Abd-El-Malek and Gibson [23] isolated *S. paracitrovorus* from 19 of 23 samples of raw milk and *S. citrovorus* from 3 samples. *S. paracitrovorus* was isolated from 5 of 54 samples of freshly pasteurized milk whereas *S. citrovorus* was not detected. When pasteurized milk was held for a time, *S. paracitrovorus* was isolated from 13 and *S. citrovorus* from 1 of 28 samples.

In some instances in which pure cultures of *S. lactis* have been carried through a series of transfers in butter plants, the cultures eventually acquired a typical culture flavor. Undoubtedly, this was due to contamination in the plants and indicates that the flavor organisms are widely distributed. It also explains why cultures of *S. lactis* can be sold as lactic cultures and satisfactory flavor obtained in a certain percentage of cases.

DEVELOPMENT OF LACTIC CULTURES

In developing lactic cultures the two types of organisms, *S. lactis* and *S. citrovorus* or *S. paracitrovorus*, can be mixed directly in sterile milk or the latter type can be grown for a few hours, or overnight, and *S. lactis* then added. When the mixed culture has developed considerable acid, it is carried through a number of transfers in sterile milk and then, when coagulation becomes rapid, it can be used in thoroughly pasteurized milk. All transfers should be grown at 70 to 72 F. Early transfers may coagulate slowly because of the restraining action of the citric acid-fermenting type on *S. lactis* and, for this reason, the interval between the inoculation of the two types should not be excessive.

The *S. lactis* culture used should be one that forms acid rather rapidly and does not produce an objectionable flavor. Farmer and Hammer [28] noted that cultures developed with *S. lactis* var. *maltigenes* had a malty flavor through a series of transfers; *S. lactis* strains pro-

ducing a flavor suggesting cabbage when grown alone in milk developed the same flavor in combination with certain citric acid-fermenting strains whereas with others they did not. An *S. lactis* strain which combines satisfactorily with one citric acid-fermenting strain may not combine satisfactorily with another.

There appears to be no basis on which flavor organisms can be selected for use in developing lactic cultures. Some of the organisms that are normal in all respects are useless for this purpose; others combine satisfactorily with only certain *S. lactis* strains. *S. citrovorus* and *S. paracitrovorus* are equally useful. Good cultures have been obtained by employing both species in combination with *S. lactis*, although there is no advantage in this.

Cultures developed from mixtures of organisms vary a great deal and the percentage that is entirely satisfactory is low. Apparently, it is difficult for the two types of organisms to establish a relationship that results in the formation of the products normally present in lactic cultures. Often the desired flavor does not appear, owing undoubtedly to failure of the flavor organisms to develop, and the culture suggests a pure culture of *S. lactis*. Some mixed cultures become satisfactory very quickly; others require a number of transfers. Unless there is an objectionable condition, or a complete lack of the desired flavor, a culture should not be considered a failure until it has been through at least 10 transfers in pasteurized milk. A culture that appears satisfactory should be carried for some time before a final decision is made with reference to it, since a stability that makes it possible to obtain good results regularly with a culture is very desirable. Use of a culture in making one or more products provides valuable information, and when the keeping qualities of such products are determined the test of the culture is complete.

COMPOUNDS FORMED DURING RIPENING OF A LACTIC CULTURE

During ripening of a lactic culture, chemical changes take place that are the result of the activity of the two types of bacteria normally present. Certain of the compounds formed account for the characteristic flavor of a culture.[29]

Lactic Acid. Lactic acid is produced in largest amount during ripening of a culture. A ripened culture contains 0.75 to 1.00%, or more, acid, calculated as lactic (*p*H 4.7 to 4.3). Lactic cultures vary considerably in the rate of acid production because of differences in

the strains of organisms present and other factors. Although lactic acid is odorless, it has a definite influence on the flavor because of its acid taste. The acid is formed largely from lactose by *S. lactis* and, accordingly, is D(+). However, Hammer [30] noted that ripened cultures contained some racemic lactic acid, the percentage of racemic acid being variable when *S. citrovorus* was associated with *S. lactis* in the culture and generally high when *S. paracitrovorus* was present. *S. paracitrovorus* definitely produces D(−) lactic acid from lactose.

Volatile Acids. The volatile acids produced in ripened culture are acetic and propionic; acetic acid is produced in the larger amount.[31] These acids influence the flavor of lactic culture and account for the distinct acid odor. A small amount of volatile acid is produced by *S. lactis* from lactose, but the larger amount is derived from citric acid by action of the flavor organisms. The amount of volatile acid produced by a lactic culture can be increased greatly by adding citric acid to the milk used. Templeton and Sommer [32] found that addition of citric acid, or sodium citrate, to the milk used for culture increased the volatile acids about 50% whereas the titratable acidity was increased not more than 10%.

Carbon Dioxide. Carbon dioxide is produced in a culture in relatively small quantities, most of it being formed by the flavor organisms. Ordinarily, the carbon dioxide remains in solution or diffuses into the air although, occasionally, a few bubbles are noted in a coagulated culture. If citric acid is added to the milk used, the carbon dioxide formed is greatly increased; through supersaturation of the fermenting milk, much of the carbon dioxide may be held in solution until the culture is stirred or otherwise agitated, when it is released rapidly. Carbon dioxide influences the flavor of a culture in much the way that it influences the flavor of carbonated beverages.

Neutral 4-Carbon Compounds. The C_4 compounds that are present in a lactic culture are acetylmethylcarbinol, biacetyl, and 2,3-butylene glycol. They are produced primarily by the flavor organisms from citric acid and their combined yield can be increased greatly by addition of citric acid.

Acetylmethylcarbinol ($CH_3 \cdot CHOH \cdot CO \cdot CH_3$) is a liquid that readily polymerizes to a solid form, especially at low temperatures; the polymerized form often persists at ordinary temperatures. Highly purified acetylmethylcarbinol is odorless but many preparations have an odor suggestive of dilute biacetyl. Acetylmethylcarbinol is important in a culture primarily because large amounts of it tend to be accompanied by comparatively large amounts of biacetyl, although

the actual quantities of biacetyl are much the smaller. Prill and Hammer [33] indicated that such factors as oxygen supply, temperature, content of citric acid, etc., prevent the establishment of any close relationship between the two compounds. Acetylmethylcarbinol is the intermediate C_4 compound from the standpoint of oxidation. Its rapid reduction to 2,3-butylene glycol under favorable growth conditions for the flavor organisms prevents its accumulation in a culture until the *p*H is comparatively low. Certain strains of *S. lactis* produce acetylmethylcarbinol from lactose and thus may be a minor factor in its production in a culture; under certain conditions, they also tend to reduce it.

Biacetyl ($CH_3 \cdot CO \cdot CO \cdot CH_3$) is a very volatile, yellow liquid with a sharp pungent odor. When sufficiently dilute, the odor is pleasing to many people. Biacetyl is the important C_4 compound because it largely accounts for the pleasing flavor of a culture and certain dairy products in which culture is used. Biacetyl is not limited to dairy products but is found in a variety of materials,[29] including raspberry juice, strawberry juice, honey, roasted coffee, bread, cane molasses, tobacco smoke, etc.; acetylmethylcarbinol also is found in various materials.

Although acetylpropionyl has an odor resembling that of biacetyl, lactic cultures apparently do not contain higher homologs of either biacetyl or acetylmethylcarbinol.[34]

2,3-butylene glycol ($CH_3 \cdot CHOH \cdot CHOH \cdot CH_3$) is an odorless compound which accumulates in a culture as a result of the reduction of acetylmethylcarbinol and biacetyl, primarily by the flavor organisms. Reduction is more rapid under conditions that favor activity of the organisms, such as a suitable temperature and low acidity. This explains the more rapid disappearance of the carbinol and biacetyl in a culture at 70 F than at lower temperatures and also the relatively rapid decrease in a neutralized culture. The glycol is the final C_4 compound formed; in a culture it apparently never is oxidized to the carbinol or biacetyl.

CONDITIONS AFFECTING PRODUCTION OF ACETYLMETHYLCARBINOL AND BIACETYL IN A LACTIC CULTURE

During the early stages of ripening, a lactic culture regularly lacks flavor. As ripening is continued and the *p*H becomes rather low, considerable flavor is produced in a short period. Continued ripening

results in a reduction of flavor, due primarily to activity of the flavor organisms.

pH. Active production of acetylmethylcarbinol and biacetyl by the flavor organisms requires a satisfactory *p*H. This can be established by other acids as well as by lactic acid. Michaelian *et al.*[35] added different acids in different amounts to pure cultures of the organisms, incubated them 48 hr at 69.8 F, and determined the acetylmethylcarbinol plus biacetyl. In general, the yield with lactic acid as the acidulant was comparatively low and a significant production occurred over a rather narrow *p*H range, maximum yields being obtained at *p*H 4.4 to 4.1. Sulfuric acid gave higher yields than lactic acid and production was significant over a wider *p*H range, with maximum yields at *p*H 3.6 to 3.2. A mixture of 0.15% lactic acid and variable amounts of sulfuric acid gave yields comparable to sulfuric acid alone, with maximum yields at *p*H 3.9 to 3.5. With citric acid the yields were much higher than with other acids and a significant production occurred over a wide *p*H range, with maximum yields at *p*H 3.9 to 3.7. The general results indicate that other acids are more satisfactory than lactic for the establishment of conditions favoring production of acetylmethylcarbinol and biacetyl by the flavor organisms.

Biacetyl production in milk by the flavor organisms was investigated by Cox[36] at *p*H values from 5.5 to 4.4, these being obtained by adding lactic acid. The organisms produced biacetyl and then partially or wholly destroyed it. The amounts produced varied with the different strains and the production capacity could not be correlated with any other property of a strain. Rate of growth of the organisms was progressively slower the lower the *p*H but at least as much biacetyl eventually was produced at a low as at a high *p*H. Barnicoat[37] reported that with a commercial lactic culture the amount of acetylmethylcarbinol plus biacetyl at 0.75% titratable acidity was only about one-third that at 0.81%.

Addition of Citric Acid. The addition of citric acid to milk for culture regularly increases the production of acetylmethylcarbinol and biacetyl during ripening, other factors being satisfactory. Laboratory results and plant experience indicate that during the ripening of a culture the flavor organisms can ferment more citric acid than normally is present in milk. The small amount of this compound naturally in milk (0.16 to 0.18%)[38,39] suggests its addition to milk intended for culture.

At a satisfactory *p*H and temperature, production of acetylmethylcarbinol and biacetyl appears to continue as long as citric acid is

present. By addition of sufficient citric acid to the original milk, the amounts of these compounds that can be formed are surprisingly large and the odor of biacetyl then is very conspicuous. Commonly, persons accustomed to this type of culture definitely prefer it to culture made without added citric acid because of the high flavor.

When citric acid, or sodium citrate, is added to milk intended for culture, an aqueous solution is prepared. Ordinarily, 0.15% citric acid (crystals containing 1 molecule of water) or 0.26% sodium citrate (crystals containing 5.5 molecules of water) is employed, although occasionally a larger amount is used. The crystals are dissolved in a small quantity of water, using heat to aid in the solution and to destroy most of the organisms present. A glass container is advisable with the acid since concentrated citric acid attacks various metals. The solution is allowed to cool and then is poured into the milk that has been pasteurized and cooled to the incubation temperature. The acid is added to the milk very slowly and with considerable agitation to prevent curdling the milk at the point where the acid strikes it. The advantage of sodium citrate is that it does not curdle milk. After the solution has been thoroughly distributed, the milk is inoculated and ripened in the usual way.

It appears that culture with a satisfactory flavor is obtained more regularly when citric acid or sodium citrate is added to the milk than when it is not. Ordinarily, the compounds are used only in large volumes of culture since their principal effect is to give more flavor rather than to influence the proportionate numbers of the two types of organisms present.

Oxygen Supply. The oxygen supply is important in the production of biacetyl. Michaelian and Hammer [40] noted that biacetyl production in acidified skim milk by pure cultures of the flavor organisms definitely was influenced by bubbling various gases through the freshly acidified cultures; oxygen regularly gave a higher yield of biacetyl than the control, through which no gas was bubbled; carbon dioxide, hydrogen, or nitrogen gave lower yields. The gases had the same general effect on production of biacetyl in lactic cultures as in the pure cultures, but the actual quantities of biacetyl formed appeared to be smaller, which may have been due to the smaller number of citric acid-fermenting organisms per milliliter. Addition of purified acetylmethylcarbinol to sterile skim milk adjusted to an acidity satisfactory for rapid production of the carbinol by the flavor organisms did not yield appreciable amounts of biacetyl in 48 to 72 hr, either with or without treatment with gas, which indicates the formation

of biacetyl from acetylmethylcarbinol in a culture is not a direct chemical oxidation but results from the action of organisms.

Brewer *et al.*[41] found that production of biacetyl in lactic cultures, and in pure cultures of the flavor organisms in milk, was greatly increased by growing the cultures under 30 lb, or more, air pressure per sq in., provided the cultures were agitated by aeration or stirring. The increase frequently amounted to several hundred per cent. In some cases the yield of acetylmethylcarbinol was increased and in others it was not. Pressure without agitation did not increase the biacetyl content. The relatively high biacetyl contents of the aerated-pressure cultures were evident in the flavor of the butter in which the cultures were used. The biacetyl content of ripened culture has been increased by shaking (aeration).[33]

Evenhuis *et al.*[42] inoculated skim milk with lactic culture and, after ripening to 0.34% acid, divided it into 5 lots. One lot (the control) was not heated and the others were heated to 104, 122, 140, and 158 F for 5 min. Biacetyl determinations were made on the heated cultures and the control before and after shaking for 30 min. All of the lots contained from 2.0 to 2.4 mg biacetyl per liter before shaking; after shaking the control culture contained 4.0 mg whereas the lots heated at 104, 122, 140, and 158 F contained 3.5, 3.1, 2.9, and 2.5 mg, respectively. The results indicate that aeration increases biacetyl formation only when the flavor organisms are viable and that the formation of biacetyl is a biological and not a chemical oxidation.

Number of Flavor Organisms Present. Since the flavor organisms are so necessary in producing the desirable compounds in a culture, their number at the time the proper *p*H is established is extremely important. If the number is too low, a culture lacks flavor. An adequate number must be built up during the early part of the ripening period so that, when the proper *p*H is reached, formation of biacetyl can occur quickly. On the other hand, an excessive number early in the ripening period is objectionable since the organisms may destroy so much of the citric acid under conditions which do not favor accumulation of biacetyl that too little remains to be fermented at a desirable *p*H. A rather delicate balance between the types of bacteria normally in a culture is very necessary for the most desirable flavor development. Addition of lactic acid to milk cultures of flavor organisms may result in larger amounts of biacetyl than are present in lactic cultures.[43]

Incubation Temperature. Lactic cultures commonly are ripened at 70 to 72 F. Dolezalek [44] noted that when cultures were ripened at

73.4 F, the formation of biacetyl first was noted after 7 to 9 hr, and a maximum was reached after 11 to 18 hr; the compound then decreased with further ripening. At temperatures above, or below, 73.4 F, production of biacetyl gradually decreased and stopped completely at temperatures above 100.4 F or below 59 F. There were two optimal temperatures for acetylmethylcarbinol production, 62.6 and 86 F. The amount of carbinol in a culture held at 73.4 F decreased after 24 hr.

Thirty-four cultures of flavor organisms studied by Folda [45] produced the most acetylmethylcarbinol at 86 F and the most acid at 73.4 F. Lactic cultures produced more flavor compounds at 71.6 than at 68 F.

Holding Ripened Cultures Cold. A desirable flavor that has developed in a culture is not maintained indefinitely. It appears that as soon as the maximum amounts of acetylmethylcarbinol and biacetyl are produced, their reduction begins, the rate being dependent on various factors. Since the reduction is due primarily to activity of the flavor organisms, it can be delayed by use of low temperatures and it is a common practice to cool ripened cultures and hold them at relatively low temperatures. With citric acid still present, cooled culture may continue to increase in acetylmethylcarbinol and biacetyl for some time. Certain butter plants find it advisable to hold ripened cultures cold for about 24 hr before use to permit an increase in biacetyl. Such an increase is made more certain by adding a small amount of citric acid to the culture at the time cooling is begun. Addition of as little as 0.08% citric acid to a ripened culture has given increases, the agitation incident to the distribution presumab'y also being a factor.[33]

Maintenance of a high biacetyl content in a ripened culture is especially important when the culture is to be added to cream at a low temperature to avoid growth of the culture organisms in the cream. Apparently, neither lactic nor volatile acids are destroyed by the normal culture organisms.

BREAKDOWN OF CITRIC ACID

The mechanism of fermentation of citric acid by the flavor organisms has been studied by several investigators. Rumments [46] suggested that the breakdown with formation of various compounds is as follows:

$$2COOH \cdot CH_2 \cdot C(OH \cdot COOH) \cdot CH_2 \cdot COOH \cdot H_2O$$

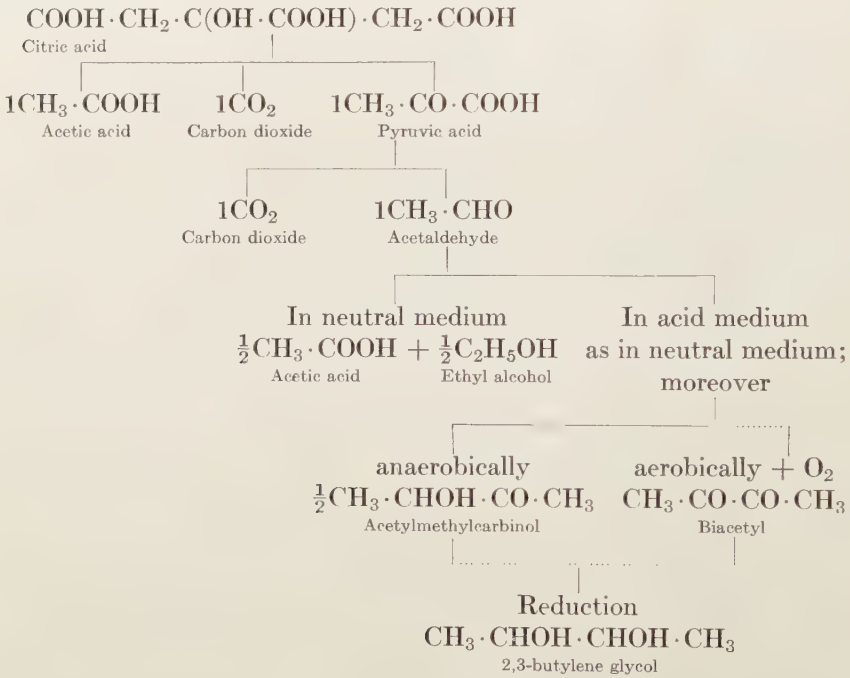
Citric acid

$$1CH_3 \cdot CHOH \cdot CO \cdot CH_3 \qquad 2CH_3 \cdot COOH \qquad 4CO_2 \qquad 2H_2O$$

Acetylmethylcarbinol — Acetic acid — Carbon dioxide — Water

The carbinol then can be oxidized to biacetyl or reduced to 2,3-butylene glycol, depending on conditions.

A more elaborate scheme for the breakdown of citric acid, in which a distinction is made between the breakdown in neutral and in acid reaction, was proposed by van Beynum and Pette [47] and is as follows:



Krenn and Valik [48] considered methyl glyoxal and α-keto glutaric acid as intermediates in the fermentation of citric acid, and Evenhuis *et al.*[49,50] reported that pyruvic acid, but not acetaldehyde, was an intermediate.

PROTEIN BREAKDOWN

Barthel and Sadler [51] found that when lactic cultures were inoculated into sterile milk containing calcium carbonate and incubated 2 months at room temperature under hydrogen or carbon dioxide, there was an increase in both the soluble and amino nitrogen. With the soluble nitrogen the increase was essentially the same as that produced by the lactic acid streptococci, but with the amino nitrogen it was much greater. Anderegg and Hammer [52] found that lactic cultures regularly proteolyzed milk, whereas some S. *lactis* cultures did and others did not. When calcium carbonate was added to the milk and distributed by occasional agitation, proteolysis was more pronounced than when it was not added. With S. *lactis*, an increase in soluble nitrogen usually was accompanied by an increase in amino nitrogen. S. *citrovorus* and S. *paracitrovorus* did not proteolyze milk. Hammer and Patil [53] reported that proteolysis was evident in freshly coagulated lactic cultures grown in pasteurized or sterilized milk. On extended holding, proteolysis was more pronounced when calcium carbonate was added to the milk than when it was not added. They also noted that S. *citrovorus* and S. *paracitrovorus* did not proteolyze milk.

Products of the protein breakdown in lactic cultures probably have little direct effect on the flavor, but they may favor growth of S. *citrovorus* and S. *paracitrovorus*. Also, desirable protein degradation in certain cheeses may be due in part to the culture organisms.

PROPAGATION OF LACTIC CULTURES

Preparation of lactic cultures is one of the very important operations in any plant using them since cultures of good quality so definitely contribute to the quality of the products in which they are employed. In making high-scoring culture butter, quality of the culture is exceeded in importance only by quality of the cream, and poor culture actually may be harmful; if contaminating organisms are present, they sometimes include species which cause deterioration in butter. In cheesemaking, use of unsatisfactory culture may result in off flavors, and, if the culture is slow in producing acid, the manufacturing operation may be delayed greatly. In culture buttermilk, flavor and body are so directly related to action of the culture used that without good culture the final product is certain to be unsatisfactory.

Success in the preparation of cultures requires attention to many details which cannot be carried out hurriedly. Care of the cultures should have precedence over all other plant operations and should not be left until there is nothing else that needs attention. If the ripening is to be controlled properly, conditions must be uniform from day to day, and when the procedures have once been standardized for a particular plant the only variable factor should be the amount of inoculation. It is especially important to inoculate the cultures at a definite time each day because intelligent variations in the amounts of inoculation can be made only when the ripening period is rather definite.

Commercial Lactic Cultures. The initial inoculum for preparation of lactic culture generally is obtained from a commercial laboratory. Such suppliers furnish cultures in either liquid or dry form. The dry form may be prepared by drying with heat or by freeze drying.

Liquid Cultures. When relatively fresh, liquid cultures contain organisms that are active and yield a satisfactory culture in a short time; their disadvantage is that they have poor keeping quality because the organisms are in a medium which supports growth and the products formed influence their activity. The usual liquid employed for commercial cultures is milk. Small volumes of milk can be sterilized in bottles and then inoculated and shipped as requests for cultures are received. Generally, the culture grows and coagulates the milk during transit. Another procedure is to transfer, under aseptic conditions, small portions of developed culture to sterile bottles. With this method, the culture is grown at a favorable temperature, instead of the varied temperatures encountered in transit, but the longer exposure of the organisms to a high acidity is a disadvantage. Addition of calcium carbonate to the bottles before sterilization has been helpful in overcoming this disadvantage. Since gas is liberated by reaction of the carbonate and the acid in the culture, magnesium oxide also has been employed but the organisms do not survive as well as with calcium carbonate.

Heat-dried Cultures. Dry cultures have good keeping quality since the organisms in them are dormant. Some dry cultures coagulate milk in a short time although with others coagulation is slow and several transfers may be necessary to produce a culture with normal activity. Dry cultures have been prepared from ripened lactic cultures by adding an inert material such as starch, lactose, milk powder, etc., to absorb a part of the water and then drying at room temperature, or slightly above, with or without vacuum. The handling

necessary in the preparation of dry cultures by certain procedures may introduce contaminants; however, the usual conditions of propagation often gradually eliminate them when the desired species begin active multiplication.

Drying kills many bacteria in a culture, but those surviving are inactive and remain so because of lack of moisture; the organisms survive for long periods, much longer than if they produced excessive acid. Rogers [54] dried lactic culture in an oven maintained at 104 to 109.4 F through which dry air was passed. From his data showing the percentage of water and the bacteria per gram at intervals during the drying, it appeared that at a water content between 5 and 10% there was a pronounced decrease in the numbers of bacteria. Rogers also dried cultures by a process in which the water was removed from the milk by a spray carried up by a current of warm, dry air; drying was complete in a short time and took place at a low temperature. Powders prepared from cultures of an active lactic acid species grown in milk showed large numbers of organisms per gram, although there was a pronounced decrease from the numbers that would have been present had there been no destruction of organisms. Data on 11 powders showed counts varying from 657,500,000 to 8,590,000,000 per g. Addition of dibasic potassium phosphate increased the total numbers of bacteria in a lactic culture, but when spray dried, unneutralized milk gave the more active powder.

Frozen-dried Cultures. Frozen-dried cultures are prepared from ripened lactic culture by freezing at subzero temperatures and drying in the frozen state under high vacuum. It was noted [54] that frozen-dried cultures were so active they curdled milk in 17 hr at 86 F when added at the rate of 1 part to 1,000,000 parts of milk. The numbers of bacteria in 11 frozen-dried cultures varied from 380,000,000 to 12,670,000,000 per g. No special advantage was gained by neutralizing cultures with calcium carbonate before drying. Also, there was no difference in activity of frozen-dried cultures prepared from skim milk or concentrated skim milk (2 to 1).

General Considerations. The investigations of Rogers [54] established a number of significant points with reference to dry cultures. Loss of activity in a powder was very slow at 32 F, or lower, and became more rapid as the temperature increased until at 86 to 98.6 F dry cultures of lactic acid bacteria became inactive in a short time. When the moisture content was comparatively high, loss of activity in powders was more rapid than when it was low. Cultures held in a vacuum retained their activity longer than cultures in an atmosphere

of nitrogen or hydrogen, and the most rapid loss of activity occurred in an atmosphere of oxygen or air.

Mother Cultures. When lactic cultures are propagated in small quantities of milk under well-controlled conditions to provide inoculating material for larger lots of milk, they are called mother cultures. Such cultures should receive special attention in an attempt to keep them vigorous and uniform, with good flavor, body, and texture. A defect in a bulk culture involves only the operations of one day, whereas a defect in a mother culture may be repeated in succeeding transfers. Ordinarily, several mother cultures are carried so that comparisons can be made and one of satisfactory quality selected.

Containers. Mother cultures are propagated in various types of containers such as milk bottles, glass fruit jars, glass-stoppered bottles, erlenmeyer flasks, and special flasks with various closures. Glass containers are advisable since they permit observations on the cultures. Small-mouth containers limit air contamination during inoculation. Plastic, or metal, screw-cap closures afford maximum protection; the cap may be loosened during heating and it is removed easily for inoculation.

Selection of Milk. Milk for mother culture or bulk culture should have a low bacterial count, be free from off flavors and odors, have an average chemical composition, be from healthy cows, and be free from substances which interfere with growth of the culture organisms.

Usually, milk with a high bacterial count cannot be freed of organisms as completely as milk with a low bacterial count and organisms which resist the heat treatment may cause abnormal conditions in cultures. Certain off flavors and odors in milk are eliminated or masked by the heat treatment but others may carry through into the ripened culture and be objectionable. Mastitis milk, which frequently has an abnormal composition, is not suitable for cultures. Milk from animals undergoing treatment for mastitis should be avoided because of the influence of various drugs in the milk on the culture organisms.

Both whole and skim milk are used in the preparation of cultures. Fat is not involved in the fermentation but gives the product a full flavor that is pleasing. Barnicoat [37] investigated the effect of the type of medium on development of acetylmethylcarbinol and biacetyl. When due allowance was made for the fat content, there was no essential difference in the action of a culture in skim milk, whole milk, and cream.

Milk reconstituted from evaporated milk ordinarily is not satisfactory for cultures; coagulation is slow, the curd is soft, and the heated

flavor is objectionable. Milk powder that reconstitutes well (low-heat powder), whether from skim milk or whole milk, often makes satisfactory culture. Horrall *et al.*[55] found that 10% spray-dried skim milk in distilled water yielded cultures as satisfactory as concentrations of 12, 15, or 18%. The activity of cultures grown in reconstituted skim milk varied less from day to day than cultures grown in fresh whole milk. Some water supplies were unsuitable for reconstituting milk for cultures and some lots of spray-dried skim milk were not satisfactory.

Knudsen and Sorensen[21] considered that the property of milk which is important in producing a satisfactory culture is the buffer action. When they obtained poor cultures, the buffer action was low and the milk might be either normal or abnormal in other respects. However, they noted that in some abnormal milk there are objectionable effects other than buffer action. Watson[56] found that Jersey milk has a markedly greater buffer capacity than Holstein milk in the *p*H zone of 5 to 6. This may explain why milk high in solids sometimes is preferred for propagating cultures.

In many plants milk for mother cultures regularly is taken from a supply that has been satisfactory over an extended period. When difficulties are experienced with the cultures, one of the first steps is to use milk from a different supply. This occasionally corrects the condition but more often it does not, which suggests that selected herd milk commonly is satisfactory for cultures.

Heat Treatment of Milk. Milk for mother cultures or bulk culture is heated to destroy most of the contained organisms and to produce a product more suitable for growth of the culture organisms. It is preferable to heat milk for mother cultures in the containers in which it is to be used. A satisfactory procedure is to place the containers, to which the milk has been added, in water reaching above the level of the milk and then heat the water with steam or in some other way. A metal box with a perforated false bottom and a cover is very useful in carrying out this method; the size is determined by the number of mother cultures and the type of container used for them. The milk should reach 180 to 200 F and be held there for at least 30 min. Then it is cooled by slowly running water into the water around the containers, care being taken to lower the temperature gradually so as to prevent breakage; slow cooling is especially necessary if the containers have thick walls.

Heating milk in flowing steam is practiced in some plants with considerable success. Milk is added to containers and placed in a

steam cabinet or autoclave where it is subjected to flowing steam for 0.5 to 1 hr. Under such conditions the milk reaches a temperature above 200 F. Sterile milk, heated for 20 min at 15 lb steam pressure, has been used for propagating cultures and all possible contamination from the milk thus is avoided. Sterile milk does not form as firm a coagulum as milk heated to lower temperatures and has considerably more heated flavor, which makes it difficult to detect slight differences in the flavors of cultures.

Inoculation of Cultures. Before inoculation, the heated milk should be adjusted to 70 to 72 F. When the inoculating material is a commercial culture, the entire culture should be used as the inoculum. If the inoculating material is a previously propagated culture, the amount of inoculum will depend upon the ripening period, activity of the culture, and accuracy of the temperature control. The common range for an incubation period of 14 to 16 hr is 0.25 to 1%.

A second mother culture is developed from the first by inoculating a small portion into milk prepared in the same way as that used for the original culture. The inoculation should be made under such conditions that contamination will not occur. Pouring over the lip of the container is not satisfactory because of the danger of adding foreign organisms and the difficulty of obtaining a definite idea of the amount of inoculating material. A satisfactory method is to use glass tubes or pipettes that have been heated with boiling water, steam, or dry heat; with these there is little chance of contamination, inoculations can be kept uniform or varied in any way that appears desirable, and cultures can be tasted by drawing small amounts into the mouth.

The series of mother cultures begun in this way is continued as long as the quality is satisfactory. Inoculations commonly are made daily, but a day can be missed occasionally without objectionable effects if the ripened cultures are kept cold. Some plants, particularly those using relatively small amounts of culture, have found it possible to limit transferring mother cultures to three, or even two, times a week and still obtain satisfactory results. Dahlberg and Ferris [57] found that, when a lactic culture was inoculated every day and also every third day and carried under excellent conditions, the quality was identical, as judged by appearance, flavor, and acid development. However, the freshly coagulated culture was somewhat less satisfactory in cheesemaking.

Incubation of Cultures. Incubation of lactic cultures generally is carried out at 70 to 72 F. If the inoculating material is a dry cul-

ture, with which coagulation sometimes is slow, incubation may be carried out at temperatures above those usually used in order to bring about more rapid coagulation. Acid development is not the only factor involved, however, and with this procedure there is the possibility that the flavor organisms in the culture will be overgrown. The temperature of the inoculated milk should be held as constant as possible.

Various methods of maintaining, or attempting to maintain, satisfactory ripening temperatures are used with mother cultures. At one extreme is the system of placing the inoculated milk in the location where the temperature is least objectionable; for example, the boiler room in winter and an open window on a warm summer night. At the other extreme are the automatically operated controllers that are heated or cooled with electricity. There are various simple systems of heating an insulated cabinet with an electric light or other resistance that are satisfactory; in summer, when room temperature is higher than the temperature desired, the cabinet can be held in a cold storage room. In many instances a well-insulated container has proved satisfactory for holding mother cultures, especially when they are surrounded with a large volume of water to delay temperature changes.

The question of sanitation should be considered with any type of incubator. Construction should be such that the equipment can be kept free of odors. A metal lining for the holding compartment is advisable and necessary if a volume of water is used to delay temperature changes; the water should be replaced frequently to avoid development of odors. Wilster and Price [58] devised a tank in which milk for mother cultures could be pasteurized and cooled, and the inoculated milk then incubated.

Under practical conditions, where the ripening temperature often is variable, satisfactory cultures may be obtained over a considerable temperature range. Toens and Hammer [59] used various temperatures and found that good cultures could be obtained as low as 64.4 F and as high as 89.6 F; at 98.6 F the flavor was very unsatisfactory and the fermented milk did not resemble a lactic culture, although sufficient acid regularly developed. Temperatures such as 70 to 72 F have advantages of considerable importance. With definitely lower temperatures growth of the organisms is delayed and it is difficult to obtain rapid ripening, whereas with significantly higher temperatures overripening often occurs and conditions are more favorable for development of heat-resistant organisms that survive pasteurization of the milk. At relatively high temperatures growth of *S. citrovorus* and

S. paracitrovorus is influenced greatly and a lactic culture that has been through a very few transfers at 98.6 F may no longer contain these organisms.

Incubation Period. The usual period of incubation for cultures which receive 0.25 to 1% inoculum, with incubation at 70 to 72 F, is between 14 and 16 hr. The culture should be firmly coagulated at the end of this period.

In some cases cultures which have lost their ability to develop a full flavor can be improved by definite overripening. Since the flavor organisms are rather tolerant of acid, continued ripening may result in this type making up a larger percentage of the total flora and thus giving more flavor in the later transfers. Overripening is the simplest method of attempting to improve cultures that lack flavor; however, extended overripening may seriously influence the activity of the culture organisms.

Cooling Cultures after Ripening. Definite coagulation is the usual basis on which a culture is cooled to limit action of the contained organisms. It can be determined without opening the container and thus without danger of contamination. Cordes and Hammer [60] noted that at low acidities the percentage of total acid made up of volatile acid was low, and, as the total acid increased, this percentage increased. When a culture is examined for flavor a decision as to whether it is overripe or underripe should be made, and, on the basis of this, the inoculation of later transfers can be adjusted. At present, cultures commonly are ripened to higher acidities than formerly.

Preparation of Large Volumes of Lactic Culture. Large volumes of lactic culture are prepared with the same general procedure that is employed for mother cultures. Commonly, the milk is pasteurized in culture tanks or vats equipped with steam and cooling water, and the ripening carried out in these. Some plant operators prefer to ripen culture in 10-gal cans. These can be placed in a tank of water and the heating, cooling, and incubation of milk carried out in the tank by controlling the temperature of the water, or the milk can be heated and cooled in a vat and then transferred to sterilized cans that are held in a tank of water to control the ripening temperature. The inoculation of a large volume of milk is made directly with the required number of mother cultures, or a special intermediate culture can be prepared in a container of suitable size. The amount of inoculating material is more difficult to determine with a large volume of milk than with a mother culture because of variations in the quantity from day to day and greater fluctuations in ripening temperature.

JUDGING LACTIC CULTURES

Anyone propagating lactic cultures should have the ability to evaluate their general quality. Both the desirable and undesirable characters should be noted each time a culture is examined. An understanding of the probable causes of various objectionable conditions is essential if the quality of the cultures is to be maintained.

General Appearance. Ripened mother cultures can be examined for their general appearance at any time, but the remainder of the examination should be delayed until transfers have been made because of the possibility of contamination. If bubbles of gas have formed they usually are present near the bottom of a culture although sometimes they are distributed throughout. Furrows made by bubbles passing to the surface sometimes are evident next to the wall of the container. Since S. *citrovorus* and S. *paracitrovorus* produce carbon dioxide from citric acid, a normal lactic culture occasionally shows a few gas bubbles because of the activity of these organisms. Gas-forming contaminants commonly give an objectionable flavor to a culture as well as produce gas bubbles in it. In some cases these organisms can be eliminated by a series of transfers, but the process is uncertain and a contaminated culture should be discarded unless the culture was at one time exceptionally good, when the normal culture organisms can be isolated and recombined. Whey at the surface of a culture is of no great significance. It may be due to high acidity, although often there is high acidity without the presence of whey. If a culture is agitated, even slightly, at about the time of coagulation, whey frequently collects.

Texture, Body, and Flavor. When a culture is examined for texture, body, and flavor, it first is thoroughly broken up by shaking or stirring. A small amount then is poured into a cup or other container and the body and texture quickly judged. The texture should be smooth and creamy, without any suggestion of lumps; there undoubtedly are various causes for an uneven texture, but one that definitely has been established is an incubation temperature that is much too high. The body should be of such a character that when the culture is poured on a surface it stands up well and has a velvety appearance. A thin body may be due to low acidity but sometimes is caused by contaminating organisms. A culture may show ropiness, and when this occurs S. *lactis* var. *hollandicus* or a ropy strain of S. *citrovorus* or S. *paracitrovorus* ordinarily can be isolated. With some cultures

ropiness appears and disappears for no apparent reason. Although pronounced ropiness in thoroughly ripened culture definitely is objectionable, slight ropiness that quickly disappears on agitation is not important. Some cultures show conspicuous ropiness at low acidities

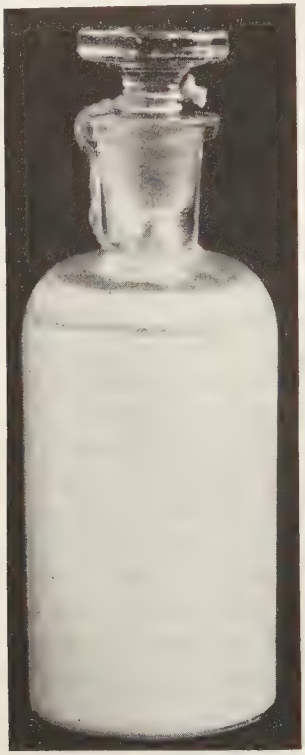


Figure 31. Mother culture showing whey beneath the cream layer.

Figure 32. Ropy mother culture.

and little or none when ripe. Occasionally, a culture has a dull, chalk-white appearance; the common cause is overripening.

The flavor of a culture should be delicate and pleasing and free from any suggestion of an objectionable condition. If a culture is insufficiently ripened it will lack flavor; if overripened the flavor will not be delicate but coarse. The decrease in acetylmethylcarbinol plus biacetyl content of a culture with extended overripening is reflected in the flavor. There is no definite acidity which always gives the most desirable flavor, and some cultures must be ripened more than others

to develop the desired condition; with a given culture there also may be variations from day to day. In some instances a culture lacks flavor because the flavor organisms develop poorly or not at all, and under these conditions no amount of ripening will give the desired flavor.

Abnormal Flavors. A number of objectionable flavors, such as barny, unclean, bitter, yeasty, rancid, etc., are encountered in cultures. These are the result either of some condition in the original milk or the activity of undesirable organisms during the ripening. Selection of the milk on the basis of freedom from off flavors is essential if a good product is to be obtained. Contaminating organisms that cause objectionable flavors in cultures come from various sources, such as unsterile transfer equipment or contamination from the mouth of the container, and when once present in a culture may be carried along from one transfer to the next. Differences in extent of their growth from day to day result in variations in intensity of the condition caused by them; the condition may even seem to disappear entirely and then later be very evident.

Conditions to Observe in Judging Cultures. In judging the flavors of cultures it is essential that the temperature be approximately uniform from day to day if accurate comparisons are to be made. Anyone accustomed to examining cultures at fairly low temperatures has difficulty in judging those that have not been cooled. When a culture is poured into a cup or other container, the flavor should be judged at once since free contact with air for only a few minutes may have a striking influence on it. There is a distinct advantage in having a number of cultures to compare, and they should differ in quality. When only one culture is examined day after day it is difficult to recognize small but significant defects, and often the culture is considered to be better than comparisons would show it to be. Another advantage is that several cultures offer more assurance of having satisfactory inoculating material since it is improbable that all the cultures will develop defects at the same time, unless there has been some irregularity in preparation.

FACTORS INFLUENCING ACID PRODUCTION BY LACTIC CULTURES

The failure of lactic cultures to produce acid uniformly and rapidly from day to day constitutes an important problem in dairy plant operations involving cultures. Slow acid production by cultures may vary from slight slowness to complete cessation of acid production.

In some cases culture failure is due to a number of factors and eliminating only one of them may not cause noticeable improvement.

Influence of Milk. Using a uniform rate of inoculation and period of incubation with lots of milk from individual animals, Baker and Hammer [61] noted that variations occurred in the acidities produced by a given culture. There was a tendency for the milk from an animal to maintain the same general ranking as to the acidity produced; in general, milk with high total solids gave a high acidity when it was made into culture, but the correlation was not a close one. Considerable variation also occurred in the flavors, and the milk from an animal fluctuated from time to time in the quality of the cultures prepared from it. Milk from various herds also showed differences in the acidities developed under definite inoculation and incubation conditions, but these were less than the differences in milk from individual animals.

Knudsen and Sorensen [21] reported that in certain cases milk from individual cows would not produce a usable culture. This sometimes was true when no disease could be detected in the animals, even on post-mortem examination, but more commonly a thorough study showed a disturbed secretion that usually was caused by an udder infection.

Milk from Infected Udders. Presumably, abnormal milk from infected udders sometimes delays the action of culture organisms, but it does not appear to be involved in typical outbreaks of slow cultures. In such outbreaks attempts to detect an unusual condition in the milk commonly are unsuccessful. Moreover, practical experience indicates that with an active culture the rate of acid production is normal in many lots of milk that include considerable volumes from abnormal udders. In this connection, Davis [62] found that unheated blood serum may inhibit certain lactic acid bacteria, for example *S. lactis*, with a small inoculum of washed cells but that under conditions similar to those in practice no effect was noticeable so that it is unlikely slow cultures are ever attributable to infiltration of serum or its constituents.

Results of various investigations [63, 64, 65] indicate that mastitis milk is not desirable for cultures. Such milk has an inhibitory effect which pasteurization only partially neutralizes. Prouty [64] noted that milk with an initial *p*H higher than 6.9 usually failed to support active growth of *S. lactis*, and adjusting the *p*H to that of normal milk only partially improved acid development in it. Addition of as little as 10% mastitis milk to normal milk retarded the growth of *S. lactis*.

Late lactation milk has an effect similar to mastitis milk,[63,65] but colostrum milk has a tendency to aid acid development.

Rancid Milk. The inhibitory effect of rancid milk on growth and acid production by *S. lactis* first was observed by Koestler [66] and has been confirmed by several investigators.[67] Tarassuk [68] noted that a small inoculation of culture into rancid milk was affected more than a larger inoculation; however, even a 5% inoculation did not produce culture of as good quality as was obtained with nonrancid milk. Tarassuk and Smith [69] found that growth of *S. lactis* was inhibited in milk with a low surface tension regardless of whether the surface tension was lowered by fat hydrolysis or addition of diglycol laurate. Costilow and Speck [70] observed inhibition of *S. lactis* in rancid milk, and the inhibition was related directly to the degree of lipolysis. However, lowering the surface tension of nonrancid milk with Tween 40 did not inhibit *S. lactis*. Later experiments [71] indicated that the inhibition was due to the toxic effect of free fatty acids; caprylic, capric, and lauric acids were most important in this respect.

Modified Milk. Various modifications of milk used for culture have been suggested, either to reduce the cost of the culture or improve its quality. Addition of lactose, fat, or water yields culture with lower acidity and less satisfactory flavor than normal milk.[61] Milk ash gives a salty taste that is not objectionable and tends to intensify the desirable flavor.

Observations by Czulak [72] suggested a loss of culture activity as a result of mutation which was enhanced by growth in a nutrient-rich medium or by a selective temperature effect. Growth of cultures in a medium rich in vitamins and other growth factors was thought to aid variants which no longer synthesized them and caused such organisms to multiply faster than other organisms which devoted part of their energy to syntheses that were no longer necessary.

Heat Treatment of Milk. The heat treatment used with milk to be made into culture influences the rate of acid development. Various investigators [63,65,73,74] have shown that lactic cultures produce acid slowly in raw milk and that pasteurizing it at 145 F for 30 min improves acid development only slightly.[73,74] Hammer and Baker [73] obtained essentially the same rate of acid development in milk heated for 30 min at 160 F as when higher temperatures were used. Foster [75] noted that acid production by *S. lactis* increased with an increase in time of exposure at 176 or 212 F. Babel [74] found that lactic cultures produced acid more rapidly in milk heated at 249 F for 15 min than in milk heated at 145, 160, or 185 F for 30 min or at 249 F for 25 min.

Several explanations have been advanced for greater acid production in heated milk. The effect may be due to the action of heat on the bactericidal property of milk.[75] Foster [75] considered the effect due to a partial hydrolysis of casein which provided the organisms with increased amounts of usable nitrogen. Analyses indicated that the nonprotein nitrogen content of milk heated at 249 F exceeded that of milk heated at 176 F by 55 to 57 γ per ml. Singh and Laxminarayana [76] were of the opinion that heat-stable, antibacterial substances were present in raw milk and to a greater extent in early lactation, late lactation, and mastitis milk in increasing order. Early lactation milk was improved considerably by heat treatment and very nearly approached normal milk in regard to acid development. Although heating improved acid production in mastitis and advanced lactation milk, such milk was quite inferior to normal milk.

Influence of Incubation Temperature. Commonly, cultures are incubated at 70 to 72 F to maintain a proper balance between the lactic acid-producing and flavor bacteria. The range of 70 to 72 F definitely is not the most favorable for acid development, and in the manufacture of various cheeses, where rate of acid increase is important, higher temperatures regularly are used. Golding *et al.*[77] found that maximum acid development occurred between 85 and 90 F; at 77 or 95 F it was about one-half that at the optimum, and at 60 or 100 F it was insignificant during 8 hr. Propagation of cultures at 70 to 72 F gave more active cultures than propagation at 86 F.[78]

The highest temperature at which lactic cultures produce acid varies with the particular culture employed. Harrison and Dearden [79] noted that one culture produced acid up to 98.6 F whereas two others produced acid at 104 F; all cultures were inactive at 113 F. Dahlberg and Ferris [57] obtained rapid acid development at 86 F and slow acid development at 100 F. With 7 lactic cultures, Babel [80] found a marked difference between acid production at 86 or 98 F and 101 F, cultures held at the latter temperature being slower. Most of the cultures produced acid very slowly at 104 F. The practice followed in cheese manufacture, whereby the milk is held for a period at about 86 F and the temperature then gradually increased to 100 to 104 F, results in greater acid development than incubation continuously at the higher temperatures.[57,80]

Influence of Overripening. Moderate overripening apparently does not influence the ability of a culture to produce acid rapidly in milk into which it is inoculated. Hammer and Baker [73] noted that holding cultures at 70 F for >30 hr after coagulation did not appreciably

decrease their activity, and Golding *et al.*[77] reported that fresh cultures were not affected by refrigeration for 24 hr. Johns and Bérard [81] found that an overripened cheese culture formed acid faster and produced cheese with a higher flavor score than the normally-ripened control culture.

By means of an activity test, Olson *et al.*[82] noted that there was an increase in the activity of cultures as the incubation time at 70 F was increased from 12 to 18 hr; the activity then remained constant during the period of 18 to 24 hr. To determine the effect of overripening for a long period, cultures were incubated regularly for 16 and 24 hr over 18 weeks. Regular overripening did not decrease the rate of acid production by cultures. In most instances the overripened cultures were the more active; they had more flavor also, but the flavor was coarser.

Influence of Air Supply. Although there is not much difference in the time of coagulation between the top and bottom of a volume of milk being made into culture, the bottom commonly coagulates first. Settling of the inoculating material may be a factor in this, but the air supply also must be considered. Toens and Hammer [59] compared the rate of acid development in thin layers of milk held in cotton-stoppered flasks with the rate in deeper layers held in glass-stoppered bottles and found that it was more rapid with a restricted than with an abundant air supply. Rice [65] noted that, at a constant temperature, aeration retards development of acid in milk by the lactic acid streptococci. However, acid formation is not the only effect to be considered, and an abundant oxygen supply tends to favor production of biacetyl, which normally is very desirable.

Katznelson and Hood [53] determined the rate of acid production by 5 cultures in pasteurized agitated and nonagitated milk. Three cultures gave lower titration values in agitated milk, but this effect could be overcome by addition of glutathione; the other 2 cultures were not affected appreciably by agitation. Raw, unagitated milk was not affected by glutathione, indicating a satisfactory reducing condition for acid production by the culture organisms. When raw milk was agitated and exposed to the atmosphere, it became a much less favorable medium for the culture organisms, a condition which was compensated for at least partly by glutathione. Freshly pasteurized milk, unagitated, and with and without glutathione, gave about the same acidity, but the same milk agitated gave a definite response to glutathione.

Milk Containing Antibiotics. The rate of growth of lactic cultures in milk containing antibiotics is influenced by the type of antibiotic, the amount present, and the sensitivity of the culture. Katznelson and Hood [84] found that penicillin was the most effective antibiotic against lactic acid-producing streptococci. It was 3.3 times as effective as aureomycin or subtilin, 6.6 times as effective as streptomycin, and 33.3 times as effective as bacitracin or chloromycetin.

Inhibitory Effect of Penicillin. The sensitivity of lactic cultures to penicillin, as determined by several investigators, is shown in the accompanying table. In general, 0.05 unit penicillin per ml causes no,

Degree of Inhibition (Units Penicillin per Milliliter Milk)				
None	Slight	Appreciable	Complete	Investigator(s)
0.05		0.15	0.30	Babel [74]
	0.05	0.10	0.50	Katznelson and Hood [84]
		0.10	0.25	Krienke [85]
0.005	0.01			Hood and Katznelson [86]
		0.10	0.25–0.3	Hunter [87]
0.05	0.05	0.11–0.16	0.32	Trembath [88]
	0.05		0.25	Scott [89]
			1.00	Jørgensen [90]
		0.10	0.50	Jepsen and Overby [91]
0.05	0.10			Dumais [92]

or very slight, inhibition of lactic cultures, 0.10 to 0.15 unit causes appreciable inhibition, and 0.25 to 0.50 unit usually causes complete inhibition.

Lactic cultures grown in whey are sensitive to smaller amounts of penicillin than when grown in milk.[84] Where a distinction is made between *S. lactis* and *S. cremoris*, *S. lactis* appears to be the more penicillin resistant. In certain instances, extremely small amounts of penicillin have actually stimulated acid production slightly.[74]

Inhibitory Effect of Aureomycin. When cows were treated with 200 mg aureomycin per quarter, Bell *et al.*[93] noted that the milk obtained 36 hr after treatment, and in some cases 60 hr, was inhibitory to lactic cultures. Autoclaving milk at 15 lb pressure for 10 min did not destroy all the aureomycin in it. Bradfield *et al.*[94] found that practically no acid developed in the milk of cows, treated with 213 mg aureomycin per quarter, for 4 milkings after treatment, and acid production was not normal until the eighth milking. Lactic cultures were not inhibited by 0.01 γ per ml but >0.1 γ gave appreciable

inhibition. Krienke [95] calculated that the aureomycin from a single infusion (200 mg) would be sufficient to inhibit acid production almost completely in 1,000 lb milk. Milk from 3 of 5 cows contained sufficient aureomycin 12 milkings after treatment to retard acid production. Johnstone [96] noted that 0.05 γ aureomycin per ml slightly inhibited lactic cultures and 1.0 γ completely inhibited them.

Extent of Antibiotics in Milk. Analyses of milk by several investigators indicate that milk from animals treated with antibiotics is not being withheld from the market. Bryan [97] found the following penicillin levels in 27 samples of herd milk selected at random in the Lansing, Mich., area: 0.00 to 0.03 unit per ml, 4 samples; 0.06 to 0.12 unit, 17 samples; and 0.25 to 0.50 unit, 6 samples. Of 1,794 samples of blended pasteurized milk obtained in New York State, Kosikowsky *et al.*[98] noted that about 7% contained inhibitory substances; 1% of them strongly inhibited lactic cultures. Johns [99] examined 642 samples of milk in the Ottawa district; 11 showed strong inhibition and 30 mild to medium inhibition of lactic cultures. In England, Storrs and Hiett-Brown [100] could not detect penicillin in 59 rail tanks or 132 vats of cheese milk, but milk from 1.4% of 712 producers in one area, 2.8% of 179 producers in another area, and 3.2% of 441 producers in still another area contained it.

Methods for Counteracting the Effect of Penicillin. Various methods have been proposed for combating the effect of antibiotics on lactic cultures. Although most of the studies have involved penicillin, some of them also apply to other antibiotics. Use of enzyme inhibitors, chemical inhibitors, reconstituted milk for culture propagation, and addition of extra amounts of culture have been suggested.

Penicillinase. Hood and Katznelson [86] added the enzyme inhibitor for penicillin (penicillinase or pennase) to milk and found that it exerted a marked effect on penicillin in a concentration of 0.05 to 0.1 unit per ml. Various bacteria produce penicillinase and can reduce the penicillin content of milk. Czekalowski [101] noted that lactobacilli, *Chromobacterium violaceum,* some micrococci, and *B. subtilis* produced the enzyme. Optimum conditions for the enzyme were 98.6 F and *p*H 7.0. Jepsen and Overby [91] found that certain coliform organisms and species of the genus *Bacillus* produced penicillinase. Addition of penicillinase did not affect the flavor of milk. Two types of penicillinase studied by Jensen and Pedersen [102] were thermostable.

Auclair [103] considered it practical to use penicillinase in the production of gruyère cheese. However, Krienke [95] believed that it is too expensive to inactivate penicillin in milk for cheese making.

Chemicals. According to Jepsen and Overby,[91] opening the β-lactan ring in the penicillin molecule, with formation of penicillinoic acid, results in complete loss of the biological activity. Such a reaction takes place on addition of various acids, alkalies, ammonia, and amines. Hydroxylamine is very active. One unit of penicillin was completely inactivated by 0.005% hydroxylamine in 1 hr. However, hydroxylamine has an inhibitory effect on the streptococci and also is toxic.

Reconstituted Milk. The use of reconstituted milk for avoiding difficulties resulting from the unexpected presence of penicillin in the fresh milk supply was recommended by Scott.[89] Previously, it was reported [55] that lactic cultures propagated in reconstituted skim milk were more uniform in activity from day to day than duplicate cultures propagated in selected herd milk.

Increased Inoculum. Krienke [95] suggested the use of an additional inoculation of cheese milk with lactic culture when slow acid production is indicated. This suggestion was based on the work of Maass and Johnson [104] who noted that each bacterial cell is capable of binding several molecules of penicillin. Thus, bacterial cells from an initial inoculation absorb considerable penicillin so that acid production does not progress at a normal rate, but, if more cells are added, there will be less penicillin to absorb and consequently the cells added later may function quite normally. Jensen and Pedersen [102] found that it was possible to maintain cultures in milk containing not more than 0.1 unit penicillin per ml by increasing the amount of inoculum; the procedure was not effective with higher concentrations.

Adaptation of Cultures. Katznelson and Hood [84] attempted to adapt 6 cultures to penicillin. Only 1 culture could be adapted and it would coagulate milk containing 2.1 units penicillin per ml. The adapted culture still was resistant to penicillin after more than 20 transfers in penicillin-free milk. By cultivating cultures in media containing penicillin, Trembath [88] also obtained some cultures with desirable cheese-making characteristics that were quite resistant to the amount of residual penicillin that might occur in milk. All cultures did not respond to this treatment. Jepsen and Overby [91] adapted a commercial culture to resist 1 unit penicillin per ml by transferring the culture 40 times in milk and increasing the amount of penicillin with each propagation. The adapted culture retained its resistance to penicillin after 30 propagations in penicillin-free milk. In the adapted culture, the flavor organisms disappeared at a penicillin concentration of 0.25 unit per ml and no biacetyl or acetylmethylcarbinol was formed by the culture.

Legislation. The Federal Security Agency considers that the direct or indirect addition of antibiotic drugs to foods for human consumption constitutes a public health hazard.[105] The reason for this is that food so treated may cause sensitization of the consumer to such antibiotics and result in the emergence of strains of pathogenic microorganisms resistant to the drugs. Foods for human consumption are considered adulterated if they contain antibiotics.

Duration of Penicillin in the Udder. The amount of penicillin injected, the type of carrier (water, ointment, etc.), and the amount of milk secreted by the animal tend to influence the duration of penicillin in the udder. With injections of 20,000 units penicillin per quarter it was noted that the average total recovery of penicillin in milk 12 hr after treatment was less with cows yielding <5 lb of milk than with those yielding >20 lb per milking.[106] Such treatment resulted in milk with 3 to 4.5 units per ml after 12 hr and 0.2 to 0.4 unit after 24 hr.[107] Foremilk contained a greater amount of penicillin than strippings 12 hr after treatment.[108] Milk from animals given 4 or 5 successive infusions contained 1.3 to 5.0 units per ml, and there appeared to be no substantial build-up as a result of the successive treatments.[109]

Hansen *et al.*[110] found that the milk from 4 cows, each treated in 1 quarter with 200,000 units of penicillin in 40 ml of a 1% solution of sulfamerazine, failed to develop sufficient acidity for cheese making until 5 or 6 milkings after treatment. Carmichael[111] noted that preparations having the same penicillin content, but different types of carriers, released from 27.5 to 81% of the penicillin into milk within 3 days. Apparently, there is no diffusion of penicillin from one quarter of an animal to another,[110, 112] and some preparations remain therapeutically effective for 3 days.[113]

Jackson and Bryan[114] determined penicillin in milk after administration of various penicillin products at various dosage levels to cows in the middle of lactation. Penicillin in water or in bougies, administered in amounts of 25,000, 50,000, and 100,000 units, could not be detected in milk after 2.5, 3, and 4 days, respectively. Penicillin in ointment base, used at the rate of 50,000 and 100,000 units, could be detected in milk after 4 but not after 5 days. Procaine penicillin in oil, administered at the rate of 300,000 units, could be detected in milk for 9 days.

The following data, obtained by Jepsen and Overby[91] show the penicillin contents of milk after various treatments and intervals.

Dose per Quarter	Number of Tests	Units of Penicillin per Milliliter of Milk after 24 hr	48 hr	72 hr
25,000	4	5.7	0.2	—
50,000	22	9.0	0.9	0.2
100,000	6	8.1	1.3	0.5

Although penicillin is administered in various dosages and some forms remain in the udder for greater periods than others, it appears that withholding the milk from treated animals for 2 or 3 days will reduce the concentration to a point where it will not cause slow acid production in mixed herd milk.

Stability of Penicillin. The stability of penicillin has been determined particularly with respect to heat. Apparently, the usual pasteurization exposures have no detectable effect on it in milk.[87,88,109] Extremely high exposures result in some destruction. For example, Hunter [109] noted that steaming milk for 1 hr, autoclaving at 10 lb pressure for 20 min, and 15 lb pressure for 15 min resulted in losses of 52, 67, and 73%, respectively. Penicillin was more stable to heat in milk than in aqueous solution. Although some loss of penicillin occurs with the heat treatment given milk intended for lactic culture, milk with a high penicillin content is not improved significantly as a growth medium by the treatment.[85,89]

Jepsen and Overby [91] found that the inhibiting effect of penicillin was decreased by holding milk. Either there was partial destruction of penicillin during storage or a certain amount of it combined with some other constituent to form an inactive compound. Loss of penicillin activity during storage of milk has been reported also by operators of cheese plants. The presence of bacteria producing penicillinase has been considered as a responsible factor.

Milk Containing Drugs and Chemicals. With certain procedures, sulfa compounds, chlorine, iodophors, quaternary ammonium compounds, etc., may be present in milk. The sulfa compounds have been used alone and in conjunction with antibiotics in the treatment of mastitis. Chlorine, iodophors, and quaternaries are used as bactericides and may be present in milk because of the failure to drain utensils treated with them or because of willful addition.

Sulfa Compounds. Krienke [95] found that 5 ml of a 25% solution of sulfamethazine added to 100 ml of milk practically prevented acid development by a lactic culture; acid development was inhibited

slightly by 0.1 ml. No sulfa drugs were detected in 922 samples of milk collected in New York State.[98]

Chlorine Compounds. Calbert [115] noted that acid production by a lactic culture in sterile skim milk was retarded by 25 to 200 ppm of chlorine. According to Miller and Elliker,[116] acid did not develop in milk containing 100 to 200 ppm chlorine when inoculated with a lactic culture or S. *cremoris* and incubated at 100 F. With incubation at 70 F, the cultures were not inhibited completely by addition of 200 ppm chlorine. Babel [117] reported that acid production by lactic cultures was slightly inhibited by 5 ppm chlorine in milk and greatly inhibited by 25 ppm.

Iodophors. An iodophor added to milk in amounts ranging from 5 to 50 ppm had no effect on acid production by lactic cultures; small quantities actually stimulated acid production.[117]

Quaternary Ammonium Compounds. Complete inhibition of acid production by lactic cultures has been reported with concentrations of 25 [116] to 50 ppm [71, 118] of quaternaries. Noticeable decreases in acid production occurred with 3,[71] 5,[116, 118] and 10 ppm.[119] Miller and Elliker [116] noted that less quaternary was required for complete inhibition at 100 F than at 70 F. Curry and Barber [118] found that concentrations of quaternary up to 25 ppm did not interfere with growth and multiplication of S. *lactis* but did block an enzyme system essential to production of lactic acid.

Kosikowsky *et al.*[98] detected quaternary ammonium compounds in about 4% of 832 samples of fresh, blended, pasteurized milk obtained throughout New York State. The concentration present ranged from a trace to 3 ppm. Milk samples containing quaternaries did not show inhibitory properties.

BACTERIOPHAGE

Bacteriophage (phage) is the term applied to bacterial viruses having the ability to lyse certain sensitive living cells. The net effect of phage on sensitive bacterial cells is destruction of the cells accompanied by an increase in the number of phage particles. Generally, the action of phage on a culture does not result in destruction of all the bacterial cells (although very nearly so), and, if the culture is held at a favorable temperature, growth will occur. Such growth probably is due to a bacterial variant which is resistant to the action of phage, and the variant is referred to as a secondary-growth organism.

Many instances of slow acid production by lactic cultures have been due to phage, and it appears that the number of culture failures due to its action is increasing. This apparent increase in phage action may be due to the ability of laboratory personnel to detect, with more cer-

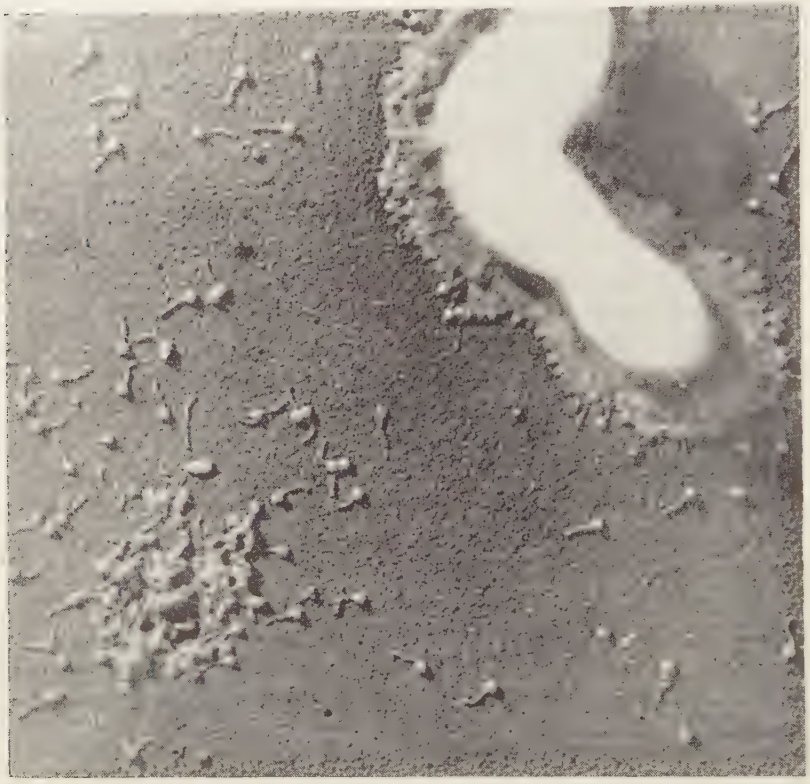


Figure 33. Bacterial cells (*Streptococcus lactis*) and phage particles. Courtesy C. E. Parmelee.

tainty, the cause of culture failure. Also, pasteurization of milk for cheese manufacture has increased considerably, and this process destroys the natural S. *lactis* flora so that the number of strains present in milk is limited to those added as lactic culture subsequent to pasteurization.

General Description. Phage particles active against S. *lactis* appear to be sperm shaped. Because of their small size, the particles generally are observed with the electron microscope although they have been seen with a regular microscope, using special technics, and with

a phase microscope.[120] Cherry and Watson[121] give the following dimensions of phage particles: Diameter of head structure 73 mμ, length of tail 160 mμ, width of tail 20 mμ, and over-all length 230 mμ. Parmelee *et al.*[122] found that phage active against *S. lactis* had a total length of 180 to 280 mμ, the heads were spherical and 60 to 90 mμ in diameter, and the tails were 20 to 40 mμ wide and 120 to 190 mμ long. The dimensions of phage particles active against *S. lactis* or *S. cremoris*, reported by several investigators, agree quite well.[121,122,123] However, Williamson and Bertaud[124] reported 1 strain

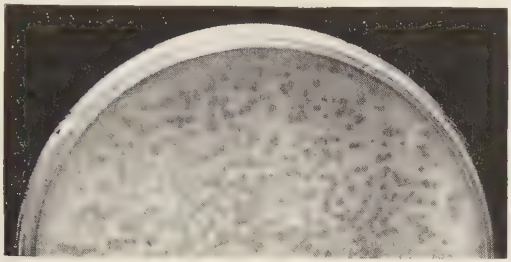


Figure 34. Section of plate showing action of bacteriophage of *Streptococcus lactis*. Courtesy R. V. Hussong.

having a spherical head 70 to 80 mμ in diameter attached to a long flagellum-like tail 15 mμ wide and 560 to 610 mμ long. Nine other phage strains examined by them had normal dimensions.

Early Investigations. In 1933, it was reported[125] that addition to pasteurized or sterilized milk of cultures that had coagulated slowly often caused considerable delay in coagulation of the milk by normal cultures; the restraining agent passed a bacteria-proof filter and was destroyed by boiling. Whitehead and Cox[126] isolated phage by the usual methods from an aerated culture which failed to develop acid. After propagation in serial culture for several weeks, it was obtained in such strength in a bacteria-free filtrate that one part of filtrate could be diluted with several hundred billion parts of milk and still lyse a susceptible organism. With the organism on a solid medium, the phage gave the classic appearance of plaques, lytic action spreading from the hypothetical phage particle to form clear spaces in the mass of confluent growth. The phage appeared to be specific in its action on one strain of *Streptococcus*.

Estimation of Phage Concentration. The number of phage particles in a bacteria-free filtrate can be estimated by serial dilution or by counting plaques (zones of lysis) on a solid or semisolid medium.

Estimation by serial dilution consists of inoculating tubes of sterile litmus milk with a sensitive culture and various dilutions of a bacteria-free filtrate containing phage. After incubation, the greatest dilution showing retarded acid production, reduction of litmus, or coagulation gives an approximation of the number of phage particles per milliliter. Plaque counts can be made by smearing a sensitive culture and a bacteria-free filtrate uniformly over the surface of a suitable solidified medium in a petri dish and incubating. Pour plates can be prepared also; with them, the sensitive culture and phage are mixed with agar and added as an overlay in a two-layer technic.

Shew [127] noted that calcium was essential in obtaining maximum plaque formation. The development of plaques in a single layer of agar medium was suggested by Yen [128] in order to obtain uniformly distributed plaques, and Gratia [129] used such a method by pouring an uninoculated agar medium into a petri dish, permitting it to harden, and then adding a mixture of agar, phage, and susceptible bacterial cells as an overlay. For enumerating phage active against *S. lactis*, Turner [130] used a base layer of 12 ml tomato juice-peptonized milk medium containing 1% agar and an overlay of a mixture of 1 ml *S. lactis* culture, 1 ml phage suspension, and 1 ml tomato juice-peptonized milk medium containing 1.5% agar and adjusted to *p*H 5.8 to 6.0. Plaque counts with this method were higher than the number of phage particles estimated by the limiting dilution method.[131] Use of 2.5% calcium chloride solution as the cell diluent increased the number of plaques [132] since phage multiplication did not occur in a calcium deficient medium.[133]

Bacteria-Bacteriophage Relationships. In the trials of Nelson *et al.*,[134] retardation of acid production often resulted when a slow culture was added to a normal culture at the time of inoculation. Plate counts showed that slow acid development was due to slow multiplication of the culture organisms. When bacteria-free filtrates from cultures were added to freshly inoculated *S. lactis* cultures or regular lactic cultures, a majority of the filtrates from slow cultures and a smaller percentage from normal cultures markedly restrained multiplication of organisms. Bacteria-free filtrates from slow and from normal cultures varied widely in their abilities to inhibit different strains of *S. lactis*. Almost every filtrate from a slow culture prevented normal acid development by at least 1 strain of *S. lactis* and most filtrates were active against several strains. Filtrates from normal cultures often contained inhibitory principle, but usually they were active against only 1 or 2 closely related organisms. It appeared that

at least part of the difference between slow and normal cultures was that slow cultures contained enough strains of inhibitory principle to inhibit a substantial fraction of the *S. lactis* types present, whereas normal cultures contained either no inhibitory principle or only 1 or 2 strains of limited activity.

According to Hunter,[135] phage active against *S. cremoris* was more or less strain specific, whereas phage active against *S. lactis* usually had a wide range of lytic activity. Overby [136] isolated phage that was relatively nonspecific; it inhibited 11 of 14 strains of organisms. Deane and Nelson [137] concluded that when a plant using a multiple-strain lactic culture experiences slow acid development due to phage, either more than one organism has been lysed or the culture contains one predominant strain. Nichols and Ineson [138] showed that one strain in a multiple-strain culture becomes predominant on prolonged daily transfer.

Hunter [139] noted that *S. cremoris* (HP) always was lysed by a specific phage race even though several phage races were capable of lysing it. Laboratory and field results suggested that the phage race active against culture HP had a special affinity for the organism and that its presence interfered with the growth of other phage races which could lyse the organism in its absence. Reiter [140] grew 4 strains of lactic streptococci separately and inoculated them into the same lot of milk for use as bulk culture. Phage which lysed one culture (HP) was detected often in the bulk culture. When each of the 4 strains was smeared on agar with 0.01 *M* calcium chloride added and the other strains spotted on it, it was evident that culture HP was an indicator strain and the other 3 strains were lysogenic or phage-carrying.

Five phage strains studied by Turner [130] proliferated by a stepwise increase, the probable burst time being about 65 min and the average burst size (average yield of phage particles per infected bacterial cell) about 90 particles. The optimum temperature for multiplication of both phage and bacteria was 89.6 F. The concentration of phage in a sensitive culture largely determines whether mass lysis will occur. Collins [141] noted that addition to skim milk of 5×10^{-3} or 5×10^{-4} phage particles per ml and 0.5% of milk cultures of host bacteria gave normal acid formation with 2 cultures. However, in the absence of evident phage action, the phage titer increased to 10^7 in 5 hr. With addition of 4.5×10^{-1} and 2.5×10^{-2} phage particles per ml and the same amount of host bacteria, mass lysis occurred. One *S. lactis* culture did not undergo mass lysis when 4% was added to

skim milk containing 25 phage particles per ml; 4 other cultures did not undergo mass lysis when 8% was added to skim milk containing 25 phage particles per ml. Mass lysis did not occur in skim milk if coagulation occurred before the phage concentration was sufficient to cause mass lysis.

Ford and Babel [142] added phage to a culture of *S. lactis* and noted that the secondary-growth organisms retained phage for 132 transfers with incubation at 69.8 F and for 10 transfers with incubation at 98.6 F. The culture was resistant to other phages as long as it retained the type which originally caused lysis. Such resistance also was noted by Czulak.[72] However, if for some reason a high phage titer is reached, the culture undergoes mass lysis. Nelson and Parmelee [143] detected phage in several commercial cultures. Such cultures appeared normal and had normal activity. Commercial cultures contained as many as 10^5 phage particles per ml but generally the count ranged from 10^2 to 10^3.

Conditions Influencing Phage Multiplication. Phage multiplication is influenced by many of the same factors which influence bacterial growth, such as composition of the medium, *p*H of the medium, and temperature. Cherry and Watson [144] found that adsorption of phage by *S. lactis* was about 3 times as great in a medium consisting of tryptone, yeast extract, and glucose as in the same medium with tryptone omitted. Electrolytes stimulated both cellular lysis and phage adsorption. Shew [127] did not obtain complete lysis of organisms in a yeast-extract lactose phosphate broth unless milk serum was added. Addition of calcium chloride had the same effect as serum, the optimum amount varying with the phosphate content of the medium. The influence of calcium chloride in promoting lysis has been reported with other media.[145,146,147]

The influence of *p*H of the medium on lysis of *S. lactis* by phage was studied by Cherry and Watson.[121] At *p*H 4, the cells were not influenced by phage or infection did not prevent some cellular multiplication. At *p*H 5, some cells probably were infected since incomplete lysis occurred at the end of the normal latent period of phage growth. The cells were completely lysed at *p*H 6, 7, and 8, but at *p*H 8 the latent period was extended. Results indicated that *p*H 5 is near the critical level below which lysis does not occur. Overcast *et al.*[148] found the optimum *p*H for phage production to be about 6.5; the 5 phage strains studied multiplied quite well over a *p*H range of 5.4 to 7.5. One phage multiplied over the *p*H range 4.8 to 9.4 while another multiplied in the range 5.2 to 7.6.

Multiplication of phage under suitable conditions must be extremely rapid to account for its effect in stopping the activity of certain cultures. Anderson and Meanwell [149] found that a minute infection of bulk culture milk with phage will increase a thousandfold during overnight incubation in the presence of susceptible culture and also that phage will retain its full strength on storage. Nelson and Parmelee [143] stated that under optimum conditions, such as *p*H 6.5, a temperature of 86 F, and a milk medium, an original inoculum of <10 phage particles per ml may increase to as many as a million per ml in 5 hr or 200 million in 7 hr.

Zehren and Whitehead [150] noted that certain cultures could not be used in cheese manufacture for more than 1 or 2 successive days without failure to produce acid, even though all practical means were taken to exclude phage contamination; other cultures were less liable to fail and could be used for longer periods. The duration of usefulness of a culture was related to the extent of air-borne phage active against the culture. Also, the extent of air contamination was related to the concentration of phage in the whey even though the culture produced acid normally during cheese manufacture. The concentration of phage in whey depended upon two characteristics of a phage race: (1) Latent period or minimum time from infection of the bacterial cell to lysis and (2) its burst size. Among 6 organism-phage combinations, the latent period varied from 40 to 90 min at 86 F and the burst size from 21 to 77 phage particles. By studying the action of different phage races on 1 bacterial strain, it was shown that both latent period and burst size were characteristics of the phage race and not of the bacterial strain.

Destruction of Phage. Various methods have been studied for destroying phage in liquids, air, etc.

Effect of Heat. Whitehead and Hunter [151] found that the thermal death points of 9 phages approximated 158 to 167 F for 30 min at *p*H 6. Cherry and Watson [121] noted that very short exposures to temperatures below 131 F had a negligible effect on phage. However, the rapid rate of phage inactivation between 131 and 149 F indicated that an important mechanism of the virus particle was being destroyed. In the studies of Miklik,[152] heating phage rapidly to 176 F or holding at 158 F for 30 min weakened it considerably and heating to 194 F or holding at 176 F for 30 min inactivated it. Novak [153] determined the effect of *p*H and temperature on phage destruction. At *p*H 8, phage was destroyed by holding at 212 F for 5 sec; at 203 F for 1 min; at 185 F for 4 min; at 167 F for 20 min; and at 149 F for 30 min.

At *p*H 6, phage was destroyed at 212 F almost instantly; at 203 F in 10 sec; at 185 F in 90 sec; at 167 F in 15 min; and at 149 F in 30 min. Nelson and Parmelee [143] stated that most phage strains survive pasteurization, and some strains tolerate 158 F for 2 hr.

Effect of Chlorine. According to Whitehead and Hunter [151] active chlorine is the most effective and convenient agent for destruction of phage. Wolf *et al.*[154] reported that fine mists containing 0.003 to 0.02% chlorine usually gave satisfactory destruction of air-borne phage; with less than about 50% relative humidity, hypochlorite solutions caused slight or negligible destruction. Parker and Elliker [155] obtained complete destruction of phage in a preparation having a titer of 10^8 per ml by using 50 ppm sodium hypochlorite and an exposure of 15 sec. Bennett [156] inactivated air-borne phage by spraying with a hypochlorite aerosol at the rate of 0.61 g available chlorine per 1,000 cu ft. In a 1 to 2 dilution of whey, 400 ppm available chlorine destroyed phage in 15 and 300 sec at *p*H 5 and 6.5, respectively. Chloramine-T was less effective than hypochlorite when used at the same concentration.

Effect of Quaternary Ammonium Compounds. Prouty [157] determined the rate of phage destruction by 6 quaternary ammonium compounds. Although some variation occurred, all of the compounds were effective. Alkyldimethylbenzylammonium chloride was the only compound that did not inactivate phage in all trials in a concentration of 100 ppm with 2- and 4-min exposures. Parker and Elliker [155] stated that the most active quaternary tested by them required a concentration of 50 ppm and an exposure time of 2 to 5 min for inactivation of phage in a preparation having a titer of 10^8 per ml. Bennett [156] noted that aerosols of quaternary ammonium compounds had an irritating effect on the respiratory tract in concentrations lower than those effective against phage. Phage in a 1 to 2 dilution of whey was destroyed by 1,000 ppm alkyldimethylbenzylammonium chloride in 30 sec at *p*H 6.6 but not at *p*H 4.6. Four other quaternaries gave similar results.

Effect of Radiations. Various quantities of a bacteria-free filtrate containing phage active against *S. cremoris* were irradiated by Sutton.[158] When the filtrates were placed 3 in. from the ultraviolet lamp, phage was completely destroyed in 6 min. Phage in milk cultures treated similarly was not affected by irradiation for 1 hr. Whitehead and Hunter [159] stated that phage active against lactic streptococci could be destroyed by irradiation provided the necessary exposure was given. However, the practical value of ultraviolet light for de-

struction of phage within the cheese plant was considered questionable because of constant reinfection occurring in the plant. Greene and Babel [160] found that the rate of destruction of phage by ultraviolet light depended upon the distance of the light from the phage, titer of the material being irradiated, energy output of the ultraviolet lamp, thickness of the film being irradiated, and whether phage was suspended in a liquid or irradiated in a dry state. The comparatively long time required for destruction of phage at short distances from a lamp, and also for destruction of dry phage, appeared to limit the practical application of ultraviolet lamps for destruction of phage in cheese plants.

Effect of Other Considerations. The inhibitory principle (phage) studied by Nelson *et al.*[154] was not affected by freezing and, at the proper *p*H, only slightly by drying. Crystal violet, methylene blue, hydrogen peroxide, and potassium permanganate inactivated it. Hunter and Whitehead [161] give the following exposures for destruction of phage by various chemicals: Hypochlorite (0.05%) <1 min, potassium permanganate (0.05%) 1 to 5 min, hydrogen peroxide (0.5%) 1 to 24 hr, formaldehyde (0.05%) 1 to 24 hr, mercuric chloride (0.05%) >14 days, alcohol (80%) 2 to 3 days, and phenol (2.5%) >14 days. Prouty [162] studied the influence of *p*H on phage to determine whether the acid and alkaline detergents used in the dairy industry would have an effect on it. Because of the tolerance of phage to both high and low *p*H levels, it was questionable whether the ordinary alkaline and acid detergents used in dairy plants would be of value in controlling an outbreak of phage infection, particularly if used at low temperatures. According to Bennett,[156] glycols are not effective against phage.

Sources of Bacteriophage. Since phage multiplies at the expense of sensitive bacterial cells, material in which there has been extensive growth of the culture organisms must be considered a possible source of phage. Rinsings from vats in which culture has been prepared, whey from cheese vats, etc., may contain it.

Phage strains active against lactic acid streptococci were found in the atmospheres of cheese factories by Whitehead and Hunter.[163] Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for air-borne phage, but whey-contaminated dust also may play a part. The concentration of air-borne phage sometimes was so great that with normal technic it was impossible to prevent infection of the cultures for more than a few transfers. Protection from air-borne phage eliminated failures due to phage.

The opportunities for phage to get into the air undoubtedly are greater in certain types of plants than in others. Perhaps they are greatest in cheese plants with milk, curd, and whey to be handled, often with considerable agitation. Open whey separators are of special importance in this connection.

Hunter [159] stated that the only sure way of isolating a fresh race of phage active against a lactic culture is to expose the culture to air in a cheese factory. Nichols and Hoyle [164] isolated phage active against lactic streptococci from bulk milk, whey, cheese, and on one occasion from pig feces. From a study of phage in a butter plant, Novak [153] concluded that the only regular source was cream inoculated with culture. The presence of phage in milk, air, and on dairy equipment was considered a secondary source. Milk drawn aseptically and the water supplies on farms did not contain phage.

Hunter [165] noted that the presence in cheese milk of phage active against lactic acid organisms used to inoculate the milk leads to its multiplication during the cheese making process. The extent to which the phage is evident in the vat depends largely on the initial infection. Whitehead and Hunter [166] stated that, when a culture is discontinued in a cheese plant and used several months later, phage generally appears in the whey on the first or second day of use. Sources of considerable phage contamination were the culture, utensils and equipment, and the milk supply. The latter source resulted from contamination by milk cans used to transport whey.

Control of Phage Outbreaks. In some instances outbreaks of slow cultures, that evidently are due to phage, rather quickly disappear without any special control procedures. Frequently, however, the defect tends to persist, with variations in degree from day to day and perhaps from week to week, unless a resistant culture is obtained; it may become so serious that tanks of buttermilk, vats of cottage cheese, etc., are lost because of the great delay in acid development. One of the confusing things in an outbreak of phage is that there may be a distinct difference in the rates of acid development in two vats of milk set with the same lot of culture.

When several cultures are carried in a plant, one or more may prove resistant to the phage that is active against others and thus provide an immediate solution of the difficulty. If this is not the case, cultures should be obtained from a source other than the one supplying those causing the irregularity. In many instances cultures from a new source quickly establish normal acid production in the plant operations. Presumably, the new cultures are resistant to the phage causing the

defect or are free of serious phage contamination and remain in that condition. Numerous outbreaks of considerable duration have been immediately terminated by bringing to the plant a culture from a new source. On the other hand, with the new cultures coming from the same source as the old ones, outbreaks have continued, even with all possible precautions in carrying the cultures. Termination of a phage outbreak by introduction of cultures from a new source is no guarantee for the future. Instances have been encountered in which the new cultures give normal acid production for an extended period and then fail. In some plants cultures from new sources are obtained rather frequently in an attempt to avoid irregularities in the culture operations. Those that are not needed immediately can be kept frozen for use in an emergency.

During an outbreak of slow acid production in cheese manufacture, Babel [167] attempted to control the slowness by use of cultures that were not sensitive to the phage types involved. Five selected cultures developed sensitivity to phage after use in cheese for only 1 or 2 days. A culture isolated from sour cream produced acid rapidly in cheese manufacture for 3 months before it developed sensitivity towards phage, and another culture, also isolated from sour cream, produced acid satisfactorily for more than 6 months; at the end of this time it was not sensitive to any phage available. Whitehead and Hunter [168] reported that, when freshly isolated strains of *S. cremoris* or *S. lactis* were used in cheese manufacture, there commonly was complete absence of a specific phage in the factory for weeks or even months. They suggested a culture rotation system for controlling phage in cheese plants in which unrelated strains of lactic streptococci would be used. Each culture in the rotation would be used every 4 days. With this procedure, there would be considerable dilution of phage within the plant because of several clean-ups and applications of bactericide before a culture was used again.

Hunter and Whitehead [169] observed that some streptococci can grow in association with certain phage races and that the presence of the phage conferred a protection against attack by other phages. This observation suggested use of phage-carrying cultures for cheese manufacture since it might protect the culture from air-borne phage. Use of such a culture in a cheese factory appeared quite promising from the practical standpoint, but soon it became evident that the culture did not retain its associated phage indefinitely and the protection offered the culture by the associated phage was not effective against all phage races capable of lysing the culture.

Nichols and Ineson [138] noted that cultures which fail to produce acid in the cheese vat may, on continued daily subculture, behave normally after a few days. The resistant secondary strains, which sometimes develop after a single strain has phaged, may produce the acidity necessary for cheese making and bring about recovery of the culture. In the early experiments with phage, resistant cultures were visualized. However, it became apparent that cultures resistant to a given phage soon were attacked by other phages.[169]

Prouty [170] attempted to attenuate phage so that it might be used to immunize cultures. Phage-infected milk heated to 158 to 167 F and held for 30 to 60 min, phage-infected whey adjusted to *p*H 2.5 to 11.8 and held for 1 to 30 min, phage-infected milk stored at 98.6 to 113 F, and phage-infected whey dried and stored in the desiccated state all failed to confer any resistance on lactic acid streptococci.

Cultures containing two, or more, strains of lactic streptococci have been considered as a possible means of reducing the effect of phage on acid production because, if only one strain was lysed, acid would be produced by the remaining strains. Collins [171] added to sterile milk a quantity of phage sufficient to give 2,500 particles per ml and equal amounts of a susceptible and a resistant strain of lactic streptococci. Acid production by the mixed culture was decreased because of mass lysis of the susceptible strain, but it was greater than that of an equal portion of the resistant culture grown separately. The effect of phage on acid production was slight where susceptible bacteria were used as 50% of the total inoculum; acid production was appreciable by mixtures containing only 5 or 10% nonsusceptible organisms. Use of equal amounts of two unrelated cultures was considered to give definite protection against culture difficulties due to phage.

A culture studied by Wagenaar and Prouty [172] did not develop phage-resistant strains when the organisms were growing actively in milk with a very low phage titer. A phage concentration sufficient to produce mass lysis was necessary for their development.

In order to minimize difficulties from slow cultures, all possible precautions should be taken in the normal handling of the cultures. Separate rooms for transferring are advisable to reduce the opportunities for entrance of phage from the air of the plant. Such rooms can be at some distance from the part of the plant in which cultures are used or in a separate building. In some instances transferring the cultures away from the usual plant operations has avoided slow cultures when previously they occurred rather frequently.

An ideal solution of the phage problem would be some treatment which would destroy any phage present in a culture and leave the organisms in an active condition. Attempts along this line, using either chemicals or heat, have been unsuccessful. In general, the phage is more resistant than the homologous organism. In this connection, Hunter [173] found that phage strains show a wider diversity of reaction to temperature conditions than the homologous organism. They frequently have different optimum growth temperatures, quite distinct from those of the substrate organism. Some strains failed to multiply at 98.6 F.

Nascent Phage Phenomenon. Certain cultures of lactic acid streptococci, normally resistant to a particular strain of phage, appear to be inhibited or lysed by the phage when a sensitive culture is introduced. This nascent phenomenon first was described by Evans [174] and since has been studied by several investigators.[119,175] Collins [176] noted that inhibition of certain heterologous strains of bacteria by phage occurred in the absence of host bacteria when very large numbers of phage particles were present. The homologous host, which previously was thought to be essential for occurrence of the nascent phenomenon, appeared necessary only as a means of increasing the number of phage particles to a point where most of the heterologous bacteria would be attacked.

Whitehead *et al.*[177] encountered instances of phage adsorption by lactic streptococci without subsequent lysis. Certain phages which exerted a lytic effect on some strains of streptococci were adsorbed by other strains without any lytic effect, and, when present in relatively high concentration, the adsorbed phage inhibited growth of the adsorbing organisms. Since the inhibitory principle was destroyed by heat coincidentally with the phage, inhibition was considered due to phage and not to some property of the *Streptococcus*.

Microscopic observations [178] on the nascent phenomenon showed that, when the phage concentration was not too high, a culture might grow and the organisms increase in number for 1 to 2 hr. Later, the organisms became irregular in shape and fewer in number. Still later, growth might occur or the organisms disappear completely, depending on the proportion of phage present or on the particular strain of organism used. Dissolution, rather than inhibition, perhaps best described the phenomenon, and there was a resemblance to lysis although the process was longer and there was no multiplication of phage. In cheese manufacture, inhibition by a heterologous phage sometimes interfered with acid production almost as much as lysis.

However, the effect depended to some extent on the stage of growth reached by a mixed culture before lysis of one of the strains took place.

CONTAMINATING ORGANISMS

Various bacteria influence the rate of acid production by *S. lactis* so that contaminating bacteria would be expected to influence the rate of acid production by a lactic culture; presumably, some contaminants would accelerate and some delay acid production. Morgan and Curle [179] found that bacteria of the *B. subtilis* type sometimes caused slow development in lactic cultures. However, a culture of *B. subtilis* studied by Rice [180] stimulated acid production by the lactic streptococci when it was growing actively in milk; the accelerated growth was considered due to proteolysis by *B. subtilis* resulting in a more available source of nitrogen than is present in fresh milk.

Streptococci Producing Inhibitory Substances

Certain lactic streptococci produce substances which inhibit the growth of other lactic streptococci and also other organisms.[181] Whitehead and Riddet [182] isolated, from certain milk supplies, a *Streptococcus* that, during its growth in milk, produced an inhibitory substance capable of delaying growth of normal lactic acid streptococci subsequently added to the milk. Milk containing this substance was referred to as "nonacid" milk. The substance, which was not destroyed by heating to 212 F for 30 min, delayed the multiplication of lactic streptococci but not the fermenting capacity of the cells.[183] It was destroyed readily by trypsin but not by pepsin. Streptococci which produced the substance have been detected rather frequently; [184] apparently, the organisms exist irregularly and in small number in the udders of some cows, in cow dung, in silage, and on the coats of animals. Hunter and Whitehead [185] noted that milk containing the inhibitory substance can cause delayed coagulation simulating that caused by phage. Generally, such milk is of poor bacteriological quality.[186]

Two strains of streptococci studied by Oxford [187] produced an inhibitory principle which was very active against *S. cremoris*, and the strains were very similar to *S. cremoris*. The name *diplococcin* was suggested for the inhibitory substance. Mattick and Hirsch [188] made attempts to concentrate the inhibitory substance produced by *S. lactis* and obtained a preparation which completely inhibited several organisms when used in considerable dilution. The inhibitory substance

produced by *S. lactis* is not identical with that produced by *S. cremoris*.[189] The chemical behavior is dissimilar and strains of *S. lactis* which inhibit *S. cremoris* and vice versa have been isolated. Some strains of *S. lactis* are inhibited by a substance which they produce, suggesting that accumulation of the substance may be one of the causes of cessation of metabolism. The substance produced by *S. cremoris* has little or no antibacterial activity against organisms not closely related to it, whereas that produced by *S. lactis* not only inhibits many streptococci but also other cocci, some aerobic and anaerobic spore-formers, mycobacteria, and some fungi; the substance is most active against *S. cremoris*. The inhibitory substance prepared from *S. lactis* is called *nisin*.[190] It differs from diplococcin by its stability in ethyl alcohol and wide range of activity against organisms. A method for nisin preparation was outlined, the yield being about 16 to 20 mg per liter of culture. In a dry state, a preparation was stable for several months. Berridge [191] purified nisin by precipitation at *p*H 6.1 from phosphate buffer. Microscopic observations showed that nisin was in the form of long filaments.

The production of inhibitory substances by 276 strains of lactic streptococci was studied by Hoyle and Nichols; [192] 220 of the strains were *S. cremoris* obtained from lactic cultures and 56 were "wild" strains isolated from naturally souring milk of poor bacteriological quality. Fifty-nine (27%) of the *S. cremoris* cultures and 19 (34%) of the "wild" strains produced substances inhibitory to other lactic streptococci. The antagonism between strains of *S. lactis* and *S. cremoris* was studied by Wilharm and Ciblis,[193] using 15 strains of *S. lactis* and 8 of *S. cremoris* isolated from lactic cultures and 45 "wild" strains of *S. lactis* isolated from milk. With 4 exceptions, *S. lactis* was not inhibited by other strains of *S. lactis* or *S. cremoris* and only 1 strain of *S. cremoris* was inhibitory to the other 7 strains. However, 40% of the strains of *S. lactis* from lactic cultures and 93% of the "wild" strains were definitely inhibitory to *S. cremoris*.

Certain strains of lactic streptococci are resistant to the inhibitory substance produced in milk by other streptococci; such cultures may or may not produce the substance. Resistant strains (classified as *S. lactis*) have been used in commercial plants for 5 yr without losing their resistance.[194]

Nisin crystallizes from cool 80% ethyl alcohol at *p*H 4.2 and inhibits growth of *S. agalactiae* and *S. cremoris* in dilutions of 10^{8} and 10^{-9}, respectively.[195] The growth medium for cultivation of nisin-producing

streptococci influences the yield of nisin.[196] Hirsch [197] reported that a single strain of *S. lactis* is capable of producing several antibiotics.

SPECIAL CULTURES FOR BUTTER MANUFACTURE

The usual lactic cultures are employed in the butter industry with very beneficial results when a product with considerable flavor is desired. One of the difficulties with them is their variability, even under well-controlled conditions. This variation is especially serious when the culture needed for the day fails to coagulate or develop the desired flavor.

Pure Cultures of *S. citrovorus* or *S. paracitrovorus*. The possibility of using a pure culture of *S. citrovorus* or *S. paracitrovorus* for development of flavor in butter has been studied by various investigators. Hammer [198] found that, with added citric or lactic acid, either *S. citrovorus* or *S. paracitrovorus* is capable of bringing about a fermentation in sweet cream that results in a desirable flavor in the butter. Maddock [199] reported that, although butter of exceptionally fine flavor can be produced with cultures of *S. paracitrovorus* under laboratory control, the uncertainties under practical conditions, owing to the slow growth, are too marked to warrant their general use.

A special type of culture in which *S. citrovorus* or *S. paracitrovorus* is grown in milk and the milk then acidified and again incubated has yielded relatively large amounts of acetylmethylcarbinol plus biacetyl.[13] The method of preparation is to inoculate either species into thoroughly pasteurized milk, allow it to grow at 70 F for 15 to 24 hr, add 0.15% citric acid and about 0.3% sulfuric acid (in mixed aqueous solution) with vigorous agitation, and then continue the incubation at 70 F for 20 to 24 hr. Before addition of the acids there commonly is no acetylmethylcarbinol plus biacetyl present, but after the *p*H is lowered formation of these materials is rapid; following the extensive production, there is a decrease unless action of the organisms is controlled by relatively low temperatures. With this method of acidification, a satisfactory *p*H for most cultures of *S. citrovorus* or *S. paracitrovorus* is from 3.9 to 4.3 although strains vary in this respect. Addition of the acids causes a rapid coagulation of the milk, and there commonly is a contraction of the curd and separation of whey. With the procedure the final products are very uniform, provided suitable cultures are used and the *p*H properly adjusted, because the growth of only one species is involved. At the Iowa Agricultural Experiment Station these cultures were found very useful for the production of

flavor in butter. The period required for their development is a disadvantage and certain plants lack facilities for maintaining pure cultures of *S. citrovorus* or *S. paracitrovorus.*

In attempts to use pure cultures of *S. citrovorus* or *S. paracitrovorus* in the manufacture of various products, it should be recognized that under certain conditions their growth may be favored by the presence of *S. lactis.* Hansen [200] found that extracts of *S. lactis* stimulated development of *S. citrovorus,* both the end point of fermentation and the final bacterial crop being increased.

Special Species. Several investigators have isolated from lactic cultures, or other dairy products, streptococci which produce relatively large amounts of lactic acid in milk as well as volatile acid, carbon dioxide, acetylmethylcarbinol, and biacetyl. In respect to sugar fermentation, the organisms resemble *S. lactis,* but the flavor compounds produced suggest a relationship to the citric acid-fermenting streptococci.

Matuszewski and associates [201] isolated an organism which they designated *Streptococcus diacetilactis* that produces considerable D(+) lactic acid and acetylmethylcarbinol in milk; the yield of carbinol is increased with added sodium citrate. They suggested this organism for use in the development of flavor in butter. Van Beynum and Pette [202] obtained an organism that rapidly produces D(+) lactic acid in milk and vigorously ferments citric acid with production of acetic acid, carbon dioxide, acetylmethylcarbinol, and biacetyl; they designated it *Streptococcus citrophilus.* Swartling [203] isolated 35 strains of streptococci which produced acetylmethylcarbinol and biacetyl; they were obtained from raw milk, lactic cultures, and dairy products. The strains showed a tendency to form a homogeneous group which resembled *S. lactis* in morphology, growth temperature, tolerance to sodium chloride, and carbohydrate fermentation. They differed from it in their ability to ferment citrate and produce carbon dioxide, volatile acids, and C_4 compounds. The strains were identical with *S. citrophilus.* It was suggested that the organisms be given species rank under the name *S. diacetilactis.*

LABORATORY EXAMINATION OF LACTIC CULTURES

In the control of the lactic cultures used in a plant, daily examinations for flavor and general condition are essential if the proper quality is to be maintained. Frequently, such examination can be advantageously supplemented with various laboratory tests.

Microbiological Examination. Cultures may contain contaminating organisms that are not evident from the flavor. These may have gained entrance during propagation in the plants or they may have been present in the cultures from the distributing laboratories. Walts [204] examined 9 cultures (7 liquid and 2 dry) from various manufacturers and found 5 of them contaminated, 4 with alkali-forming bacteria, and 1 with yeasts. Laboratory examination of cultures being carried under plant conditions indicate that contaminating organisms rather frequently are present, usually in small numbers.

The organisms contaminating cultures include bacteria, yeasts, and molds. In some cases foreign bacteria can be eliminated from a culture by exposing them to high acidities through overripening, but such a procedure is not effective with certain organisms, especially yeasts and molds, because of their acid tolerance. The mold most frequently found in cultures is *G. candidum.*

Organisms that differ in morphology from the normal culture species can be detected by microscopic examination of stained preparations. Molds, yeasts, and certain bacteria, when present in relatively large numbers, are shown in this way. Cultures on agar also are useful in detecting contamination. They can be prepared in plates or on agar slopes, using tomato agar or some other medium; acidified potato dextrose agar (*p*H 3.5) can be employed as a selective medium for molds and yeasts. Cultural methods permit examination of relatively large amounts of material and constitute a much more severe test than microscopic examinations.

Chemical Examination. Under certain conditions it may be advisable to determine the acetylmethylcarbinol plus biacetyl in a culture, or the biacetyl can be determined separately. Various colorimetric procedures have been suggested for such determinations.[205]

The creatine test, for detecting acetylmethylcarbinol in bacterial cultures, has been adapted to the examination of lactic cultures and gives useful information on the general content of the carbinol plus biacetyl. It is carried out as follows: [206] 2.5 ml of the culture is placed in a test tube having an inside diameter of about 0.5 in. and a small amount of creatine (a portion of the powder equal to about one-third of the volume of a wheat grain) added; the creatine can be handled best on the point of a small knife. Strong sodium hydroxide (40%) equal in volume to the volume of culture then is added to the tube, the mixture thoroughly shaken, and allowed to stand. If relatively large amounts of acetylmethylcarbinol plus biacetyl are present in the culture, a red color develops at the surface of the mixture in a few

minutes and soon extends to a depth of 0.25 in. or more. With smaller amounts of carbinol plus biacetyl, the red color develops more slowly and the colored portion is comparatively shallow. Cultures that are decidedly lacking in flavor may show no red color even after holding the test mixture for hours. When a strong reaction is obtained, the material adhering to the wall of the tube above the surface of the liquid is strikingly red in color.

The creatine test has been modified by the addition of a small amount of α-naphthol.[207,208] King [209] determined the acetylmethylcarbinol plus biacetyl content of cultures by adding 1 ml of 30% potassium hydroxide and 1 ml of a solution containing 0.1 g creatine, 4.0 g α-naphthol, 10 g amyl alcohol, and 50 g ethyl alcohol, to 2 ml culture. The mixture is adjusted to 86 F and repeatedly shaken for 30 min. Then, it is filtered through paper. A red-lilac color develops, the intensity depending on the amount of carbinol plus biacetyl.

Culture Activity Tests. Frequently, it is desirable to make comparisons between lactic cultures as to the rate of acid production. Several tests have been suggested for this, and some are important in predicting acid development in a particular manufacturing operation, such as cheese manufacture. Activity tests do not take flavor development into consideration and, therefore, do not indicate the suitability of cultures for such products as cultured buttermilk or butter.

Whitehead and Cox [210] developed a method of testing culture activity which simulated, to some extent, the cheese making process. The test was carried out in pint jars and consisted of determining the whey acidity at intervals. Johns and Berard [81] modified the procedure; they inoculated sterile skim milk with 1% culture, incubated it at 86 F for 2 hr and then at 102 F for 4 hr, and titrated the acid each hour. A similar procedure was used by Anderson and Meanwell [149] except that the milk was incubated for 6 hr at 86 and/or 98.6 F. Horrall and Elliker [211] determined culture activity in reconstituted skim milk (10%). For the test, 10 ml of sterile milk in a screw-cap test tube was inoculated with 0.3 ml culture and incubated at 100 F for 3.5 hr. After incubation, the entire contents of the tube plus 5 ml water for rinsing was titrated with 0.1 *N* sodium hydroxide. A culture developing 0.35% acid usually was active in the cheese vat provided certain other factors were constant. Golding and McCorkle [212] also used reconstituted skim milk for activity tests. The milk (100 g) and culture (1 g) were weighed for the test since a volume measurement of culture often varied as much as 10%. Incubation was at 86 F for 6 hr, and the acid formed was determined by titration.

Leber [213] suggested an activity test based on the reduction time of resazurin. Johns [214] criticized the test because it failed to show differences comparable to acid production. Golding [215] found that methylene blue or resazurin reduction was not suitable for measuring culture activity because considerable reduction occurred in milk containing growth inhibitors.

Since increases in titrable acidity, or changes in *p*H, produced by cultures in 2 to 2.5 hr are too small to be measured unless a large inoculation is used, Golding [215] suggested an activity test based on the rate of coagulation of milk by rennet. The test is conducted by adding 250 ml pasteurized milk to a pint fruit jar, adjusting to 86 F, and inoculating with 2.5 g culture. After incubation at 86 F for 2.5 hr, 10 ml dilute rennet is added and mixed. The rate of coagulation is determined with a special apparatus.

Attempts to Increase Culture Activity. Various substances have been added to milk to promote growth or acid production by lactic cultures. Hadzi-Antie [216] found that degenerated types of lactic acid streptococci could be activated by repeatedly transferring them in milk containing 1 to 100 mg per ml of potassium metabisulfite. Storrs and Anderson [217] increased culture activity by addition of 0.3 to 0.4% dry yeast-extract solids. They also prepared a stimulating growth factor for fast and slow cultures from defatted fish meal. Katznelson and Hood [218] found that addition of cysteine to pasteurized milk at the rate of 2.5 mg per 100 ml caused an increase in titratable acidity of 0.07% above the control, whereas glutathione gave an increase of 0.09%. Cystine, sodium thioglycollate, and vitamin C were only slightly stimulatory, and methionine exerted no effect. Glutathione exerted a stimulating effect in bottles of culture that were not opened or agitated, although this was not the case with the other reducing agents. Ten mg of glutathione per 100 ml milk appeared to be the optimum amount as 5 mg gave about half the effect, 15 mg produced no effect, and 20 mg gave an actual inhibition of acid production. Cysteine in an amount greater than 2.5 mg per 100 ml milk also caused inhibition. Results indicated clearly that glutathione exerts its effect primarily at the initial stage of development.

The addition of materials to milk to increase culture activity appear to have little practical value. The cost of the suggested growth stimulants prohibits their use in large quantities of milk.

REFERENCES

1. *Conn. (Storrs) Agr. Expt. Sta., 2nd Ann. Rept.*, 52 (1889).
2. *Conn. (Storrs) Agr. Expt. Sta., 3rd Ann. Rept.*, 136 (1890).
3. *Conn. (Storrs) Agr. Expt. Sta., 9th Ann. Rept.*, 17 (1896).
4. *J. Dairy Research*, **2**, 137 (1931).
5. *Milch-Ztg.*, **26**, 590 (1897).
6. *Conn. (Storrs) Agr. Expt. Sta., 11th Ann. Rept.*, 85 (1898).
7. *Ia. Agr. Expt. Sta. Research Bull.* 55 (1919).
8. *Beretning* 102, *K. Veterinaer-og landbohøjskole, Landøkonomiske forsøgslaboratorium, Copenhagen*, 1919.
9. *Centr. Bakteriol. Parasitenk.*, Abt 2, **49**, 373 (1919).
10. *Ia. Agr. Expt. Sta. Research Bull.* 63 (1920).
11. *Biochem. Z.*, **210**, 234 (1929).
12. *Biochem. Z.*, **216**, 330 (1929).
13. *Ia. Agr. Expt. Sta. Research Bull.* 155 (1933).
14. *J. Dairy Sci.*, **34**, 374 (1951).
15. *Indian J. Vet. Sci.*, **6**, 141 (1936).
16. *J. Council Sci. Ind. Research*, **12**, 232 (1939).
17. *J. Council Sci. Ind. Research*, **12**, 239 (1939).
18. *Z. physiol. Chem.*, **176**, 282 (1928).
19. *J. Bacteriol.*, **12**, 333 (1926).
20. *Ia. Agr. Expt. Sta. Research Bull.* 81 (1923).
21. *K. Veterinaer-og landbohøjskole Aarsskrift*, 64 (1929).
22. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 167 (1930).
23. *J. Dairy Research*, **15**, 233 (1948).
24. *Neth. Milk Dairy J.*, **4**, 274 (1950).
25. *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, **14**, 1 (1948).
26. Benchetrit, I. *Studies on the Isolation of the Citric Acid-Fermenting Streptococci from Butter Cultures.* Thesis. Ia. State College (1933).
27. *Proc. 35th Ann. Meeting Western Div., Am. Dairy Sci. Assoc.* (1954).
28. *Ia. Agr. Expt. Sta. Research Bull.* 146 (1931).
29. *J. Dairy Sci.*, **26**, 83 (1943).
30. *Ia. Agr. Expt. Sta. Research Bull.* 65 (1920).
31. *Ia. Agr. Expt. Sta. Research Bull.* 80 (1923).
32. *J. Dairy Sci.*, **12**, 21 (1929).
33. *J. Dairy Sci.*, **22**, 67 (1939).
34. *Ia. Agr. Expt. Sta. Research Bull.* 268 (1939).
35. *J. Dairy Sci.*, **21**, 213 (1938).
36. *J. Dairy Research*, **14**, 28 (1945).
37. *J. Dairy Research*, **8**, 15 (1937).
38. *Ia. Agr. Expt. Sta. Research Bull.* 90 (1926).
39. *J. Dairy Research*, **3**, 1 (1931).
40. *Ia. Agr. Expt. Sta. Research Bull.* 205 (1936).
41. *Ia. Agr. Expt. Sta. Research Bull.* 233 (1938).
42. *Neth. Milk Dairy J.*, **5**, 110 (1951).
43. *Ia. State Coll. J. Sci.*, **18**, 267 (1944).
44. *Sborník Československ. Akad. Zemědělské*, **24**, 355 (1951).
45. *Milchwissenschaft*, **7**, 49 (1952).
46. *Pētījumi par pienskābes dīglu tīrkultūrām un to ražoto acētilmetilkarbinolu.* Rīgā, Latvijas Farmaceitu Biedrība un Latvijas Aptiekaru Biedrība. 1936.
47. *J. Dairy Research*, **10**, 250 (1939).
48. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 516 (1949).
49. *Neth. Milk Dairy J.*, **5**, 201 (1951).
50. *Neth. Milk Dairy J.*, **6**, 137 (1952).

51. *Trans. Roy. Soc. Can.*, **22** (Sec. 5), 233 (1928).
52. *J. Dairy Sci.*, **12**, 114 (1929).
53. *Ia. Agr. Expt. Sta. Research Bull.* 123 (1930).
54. *J. Infectious Diseases*, **14**, 100 (1914).
55. *J. Dairy Sci.*, **33**, 250 (1950).
56. *J. Dairy Sci.*, **14**, 50 (1931).
57. *J. Dairy Sci.*, **28**, 771 (1945).
58. *J. Dairy Sci.*, **20**, 443 (1937).
59. *Ia. Agr. Expt. Sta. Research Bull.* 85 (1925).
60. *Ia. Agr. Expt. Sta. Research Bull.* 66 (1921).
61. *Ia. Agr. Expt. Sta. Research Bull.* 92 (1926).
62. *J. Dairy Research*, **10**, 196 (1939).
63. *Dairy Inds.*, **5**, 212 (1940).
64. *J. Dairy Sci.*, **23**, 899 (1940).
65. *J. Australian Inst. Agr. Sci.*, **8**, 28 (1942).
66. *Schweiz. Milchztg.*, No. 103, Dec. 1928.
67. *Landwirtsch. Jahrb. Schweiz*, **42**, 937 (1928).
68. *Assoc. Bull. Intern. Assoc. Milk Dealers*, 32nd Yr., No. 6, 153 (1939).
69. *J. Dairy Sci.*, **23**, 1163 (1940).
70. *J. Dairy Sci.*, **34**, 1119 (1951).
71. *J. Dairy Sci.*, **34**, 1104 (1951).
72. *Australian J. Dairy Technol.*, **7**, 14 (1952).
73. *Ia. Agr. Expt. Sta. Research Bull.* 106 (1928).
74. *Purdue Agr. Expt. Sta. Bull.* 573 (1952).
75. *J. Dairy Sci.*, **35**, 988 (1952).
76. *Indian J. Dairy Sci.*, **1**, 78 (1948).
77. *J. Dairy Sci.*, **26**, 909 (1943).
78. *Proc. 32nd Ann. Meeting Western Div.*, Am. Dairy Sci. Assoc. (1951).
79. *J. Dairy Research*, **12**, 35 (1941).
80. *J. Dairy Sci.*, **29**, 589 (1946).
81. *J. Dairy Research*, **13**, 127 (1943).
82. *Okla. Agr. Expt. Sta. Bull.* B-446 (1955).
83. *Can. Dairy Ice Cream J.*, **29**, 7, 27 (1950).
84. *J. Dairy Sci.*, **32**, 961 (1949).
85. *Milk Plant Monthly*, **38** (12), 44 (1949).
86. *Can. Dairy Ice Cream J.*, **28** (3), 32 (1949).
87. *J. Dairy Research*, **16**, 39 (1949).
88. *Australian J. Dairy Technol.*, **5**, 143 (1950).
89. *Dairy Inds.*, **15**, 1260 (1950).
90. *Nord. Veterinaermed.*, **3**, 203 (1951).
91. *Neth. Milk Dairy J.*, **5**, 157 (1951).
92. *Quebec Lait*, **10** (3), 14 (1951).
93. *J. Dairy Sci.*, **34**, 675 (1951).
94. *J. Dairy Sci.*, **35**, 51 (1952).
95. *Butter, Cheese, Milk Products J.*, **41** (7), 32 (1950).
96. *Soc. Am. Bacteriologists, Proc.*, 26 (1950).
97. *Mich. Agr. Expt. Sta. Quart. Bull.*, **33**, 223 (1951).
98. *J. Dairy Sci.*, **35**, 533 (1952).
99. *Can. J. Agr. Sci.*, **33**, 586 (1953).
100. *J. Dairy Research*, **21**, 337 (1954).
101. *J. Pathol. Bacteriol.*, **62**, 85 (1950).
102. *Beretn. Forsøgsm.*, Kbh. 64 (1951).
103. *Lait*, **31**, 121 (1952).
104. *J. Bacteriol.*, **57**, 415 (1949).
105. *Federal Register*, Feb. 25, 1953.
106. *J. Comp. Pathol. Therap.*, **58**, 232 (1948).
107. *Schweiz. Arch. Tierheilk.*, **90**, 685 (1948).
108. *Can. J. Comp. Med. Vet. Sci.*, **13**, 127 (1949).
109. *J. Dairy Research*, **16**, 235 (1949).
110. *Proc. 31st Ann. Meeting Western Div.*, Am. Dairy Sci. Assoc. (1950).
111. *Vet. Record*, **61**, 886 (1949).

112. *Nord. Veterinaermed.*, **2**, 591 (1950).
113. *Nord. Veterinaermed.*, **2**, 765 (1950).
114. *Vet. Med.*, **45**, 395 (1950).
115. *Butter, Cheese, Milk Products J.*, **42** (3), 30 (1951).
116. *J. Dairy Sci.*, **34**, 279 (1951).
117. *J. Dairy Sci.*, **37**, 635 (1954).
118. *J. Milk and Food Technol.*, **15**, 278 (1952).
119. *Svenska Mejeritidn.*, **44**, 193 (1952).
120. *J. Bacteriol.*, **53**, 781 (1947).
121. *J. Bacteriol.*, **58**, 601 (1949).
122. *J. Bacteriol.*, **57**, 391 (1949).
123. *Australian J. Dairy Technol.*, **5**, 99 (1950).
124. *J. Bacteriol.*, **61**, 643 (1951).
125. *Ia. Agr. Expt. Sta. Rept. Agr. Research* 63 (1933).
126. *New Zealand J. Sci. Technol.*, **16**, 319 (1935).
127. *Nature*, **164**, 492 (1949).
128. *Proc. Soc. Exptl. Biol. Med.*, **32**, 1006 (1935).
129. *Ann. inst. Pasteur*, **57**, 652 (1936).
130. *Ia. State Coll. J. Sci.*, **24**, 112 (1949).
131. *J. Dairy Sci.*, **34**, 754 (1951).
132. *J. Bacteriol.*, **64**, 105 (1952).
133. *J. Bacteriol.*, **64**, 113 (1952).
134. *Ia. Agr. Expt. Sta. Research Bull.* 256 (1939).
135. *J. Hyg.*, **45**, 307 (1947).
136. *Maelkeritidende*, **62**, 809 and 829 (1949).
137. *J. Dairy Sci.*, **35**, 668 (1952).
138. *J. Dairy Research*, **15**, 99 (1947).
139. *J. Dairy Research*, **16**, 374 (1949).
140. *Nature*, **164**, 667 (1949).
141. *J. Dairy Sci.*, **34**, 894 (1951).
142. *J. Dairy Sci.*, **33**, 466 (1950).
143. *Milk Ind. Foundation, Conv. Proc.*, **4**, 46 (1950).
144. *J. Bacteriol.*, **58**, 611 (1949).
145. *J. Bacteriol.*, **60**, 533 (1950).
146. *Ia. State Coll. J. Sci.*, **25**, 185 (1951).
147. *Soc. Am. Bacteriologists, Proc.*, 49 (1951).
148. *J. Bacteriol.*, **61**, 87 (1951).
149. *J. Dairy Research*, **13**, 58 (1942).
150. *J. Dairy Sci.*, **37**, 209 (1954).
151. *J. Dairy Research*, **10**, 403 (1939).
152. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 551 (1949).
153. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 562 (1949).
154. *J. Dairy Research*, **14**, 291 (1946).
155. *J. Milk and Food Technol.*, **14**, 52 (1951).
156. *Ia. State Coll. J. Sci.*, **26**, 169 (1952).
157. *J. Milk and Food Technol.*, **12**, 214 (1949).
158. *J. Australian Inst. Agr. Sci.*, **7**, 67 (1941).
159. *J. Dairy Research*, **14**, 64 (1945).
160. *J. Dairy Sci.*, **31**, 509 (1948).
161. *J. Dairy Research*, **11**, 63 (1940).
162. *J. Milk and Food Technol.*, **13**, 329 (1950).
163. *J. Dairy Research*, **12**, 63 (1941).
164. *J. Dairy Research*, **16**, 167 (1949).
165. *J. Dairy Research*, **13**, 294 (1944).
166. *J. Dairy Research*, **15**, 112 (1947).
167. *J. Dairy Sci.*, **30**, 507 (1947).
168. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 629 (1949).
169. *J. Dairy Research*, **16**, 368 (1949).
170. *Proc. 32nd Ann. Meeting Western Div.*, Am. Dairy Sci. Assoc. (1951).
171. *J. Dairy Sci.*, **35**, 381 (1952).
172. *J. Dairy Sci.*, **31**, 859 (1948).
173. *J. Dairy Research*, **13**, 136 (1943).
174. *U. S. Public Health Repts.*, **49**, 1386 (1934).
175. *J. Dairy Research*, **14**, 81 (1945).
176. *J. Dairy Sci.*, **35**, 371 (1952).
177. *J. Gen. Microbiol.*, **6**, 21 (1952).
178. *J. Dairy Research*, **20**, 60 (1953).
179. *New Zealand J. Agr.*, **37**, 305 (1928).
180. *Dairy Inds.*, **14**, 498 (1949).

181. *J. Bacteriol.*, **16**, 321 (1928).
182. *New Zealand J. Agr.*, **46**, 225 (1933).
183. *Biochem. J.*, **27**, 1793 (1933).
184. *New Zealand J. Agr.*, **52**, 38 (1936).
185. *J. Dairy Research*, **13**, 123 (1943).
186. *Proc. Soc. Agr. Bacteriologists*, 19 (1943).
187. *Biochem. J.*, **38**, 178 (1944).
188. *Nature*, **154**, 551 (1944).
189. *Proc. Soc. Appl. Bacteriol.*, 26 (1946).
190. *Lancet*, **253**, 5 (1947).
191. *Lancet*, **253**, 7 (1947).
192. *J. Dairy Research*, **15**, 398 (1948).
193. *Milchwissenschaft*, **6**, 51, 87 (1951).
194. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 432 (1949).
195. *Biochem. J.*, **45**, 486 (1949).
196. *J. Gen. Microbiol.*, **5**, 208 (1951).
197. *Nature*, **167**, 1031 (1951).
198. *Ia. Agr. Expt. Sta. Research Bull.* 67 (1921).
199. *J. Dairy Research*, **2**, 203 (1931).
200. *J. Dairy Sci.*, **24**, 969 (1941).
201. *Roczniki Nauk Rolniczych i Lésnych*, **36**, 1 (1936).
202. *Verslag. Landbouwk. Onderzoek.*, **42**, 361 (1936).
203. *J. Dairy Research*, **18**, 256 (1951).
204. *Ark. Agr. Expt. Sta. Bull.* 249 (1930).
205. *Ia. State Coll. J. Sci.*, **12**, 385 (1938).
206. *J. Dairy Sci.*, **18**, 579 (1935).
207. *Proc. World's Dairy Congr., 11th Congr.*, **2**, 104 (1937).
208. *Z. Lebensm. Untersuch. u. Forsch.*, **86**, 19 (1943).
209. *Dairy Inds.*, **13**, 860 (1948).
210. *New Zealand J. Sci. Technol.*, **13**, 304 (1932).
211. *J. Dairy Sci.*, **33**, 245 (1950).
212. *Intern. Dairy Congr., Proc. 13th Congr.*, **3**, 1209 (1953).
213. *Milk Plant Monthly*, **39** (9), 40 (1950).
214. *Can. J. Agr. Sci.*, **33**, 586 (1953).
215. *Proc. 22nd Ann. State Coll. Wash. Inst. of Dairying*, 53 (1953).
216. *Milchwissenschaft*, **3**, 260 (1948).
217. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 605 (1949).
218. *Can. Dairy Ice Cream J.*, **29** (7), 27 (1950).

17 Bacteriology of Fermented Milk Preparations

Milk that has undergone extensive changes as a result of growth of selected micro-organisms is consumed in all countries in which milk is readily available. The products have a high food value and, in addition, some of them appear to have therapeutic properties, especially in certain intestinal disorders. Their use is increasing in many parts of the United States and in some localities the fermented milk sold makes up a considerable percentage of the total milk sales.

There are two main types of fermentations used with fermented milk, an acid fermentation and a combined acid and gassy fermentation. The former is employed in various types of products and the latter in such products as kefir and kumiss.

Buttermilk. Buttermilk, in the strict sense, is the material remaining when the fat is gathered in the process of butter making. If the cream that is churned has undergone a clean, acid fermentation, the buttermilk has a flavor that is pleasing to many people. If the cream is old and has undergone a variety of fermentations, the flavor of the buttermilk is unsatisfactory, especially when the cream has been treated with neutralizer. Pasteurization of sour cream usually causes the casein to harden so that, when the buttermilk obtained from it is allowed to stand, the casein settles, leaving a more or less clear whey-like material at the surface.

Because of the difficulty of obtaining buttermilk of high quality from the cream delivered to many creameries, various products are made from fresh or reconstituted skim milk, or fresh whole milk, and sold as *cultured, skim milk, or cream* (2% fat) buttermilk,[1] or under some special name adopted by the manufacturer. Whether buttermilk is made from skim, part skim, or whole milk depends on the price for which it is to sell. For many consumers the presence of fat gives a more pleasing product. Burke [2] stated that his selection of materials for preparation of cultured buttermilk, in order of preference, is: (1) Milk containing about 4% fat, (2) part skim milk containing 1.5 to 2.0% fat, (3) skim milk to which cream is added later, (4) reconstituted milk, and (5) condensed milk. Occasionally, butter granules are added to cultured buttermilk to give it an attractive appearance or cream is added and granules produced by churning.

In the preparation of cultured buttermilk the fermentation commonly is carried out with lactic culture, and the important factors in production of acid, flavor, etc., are essentially the same as with lactic culture (see Chapter 16). The acidity developed in cultured buttermilk must be sufficiently high for the formation of flavor compounds (biacetyl, volatile acids, carbon dioxide, etc.). Schock and Breazeale [3] found that cultured buttermilk manufactured from skim milk containing 8.86% solids and incubated to yield 0.73, 0.79, and 0.85% titratable acidity gave values for acetylmethylcarbinol plus biacetyl (nickel salt per 200 g) of 0, 3.1, and 21.8 mg, respectively, when fresh; after holding cold for 48 hr, the values were 5.1, 14.9, and 25.1, and after 72 hr the values were 9.5, 16.7, and 21.8 mg. Wilkowske [4] noted that when the titratable acidities of cultured buttermilk made from skim milk containing 9 and 11% solids were both 0.76%, the *p*H of the latter was almost 0.25 unit higher. He considered that a cultured buttermilk with 11% solids should show 0.12% more acid than a product containing 9% solids for comparable flavor development. Glenn and Prouty [5] found that very little acetylmethylcarbinol plus biacetyl was present in cultured buttermilk prior to coagulation. However, after coagulation, the rate at which these compounds were produced increased rapidly, and rapid production continued until the incubation time was 24 hr. Later, there was destruction of the flavor compounds. The lactic acid-producing bacteria made up 90 to 99% of the total flora of cultured buttermilk, and their maximum number was reached in 15 to 20 hr with incubation at 71.6 F. The citric acid-fermenting bacteria increased in number with incubation at 71.6 F for 35 hr or more. At the time of coagulation these organisms made up 1.0 to

2.79% of the total flora, and after incubation for 24 hr they equaled 3.4 to 5.29% of the flora.

Since some people prefer cultured buttermilk rather low in acidity and a fairly high acidity is necessary for proper flavor development, the acidity can be reduced after the fermentation is complete by adding pasteurized sweet skim or whole milk and thoroughly agitating. This procedure also reduces the viscosity of the product. A satisfactory ratio is 1 part skim or whole milk to 2 parts of fermented milk, but this can be varied widely.

The importance of citric acid as a source of flavor compounds in lactic culture has led to its use in making cultured buttermilk. Nelson [6] reported that addition of 0.15% citric acid crystals, dissolved in distilled water, to pasteurized skim milk to be made into buttermilk regularly gave the finished product an improved flavor. The creatine test showed that the added citric acid resulted in more acetylmethylcarbinol plus biacetyl in the product. Addition of 0.15% sodium citrate to skim milk used for preparing buttermilk also has given marked increases in flavor compounds.[3]

Manufacture of Cultured Buttermilk from Reconstituted Skim Milk. Because in certain areas the supply of fresh milk is limited, cultured buttermilk often is manufactured from reconstituted (low heat) skim milk, using about 10% solids.[3,7,8,9] Lower solids (8%) may give a product with a thin body and higher solids (11%) a product with too heavy a body.[9] Different lots of powder used for reconstitution show variations in quality of the finished product; thin body, grainy texture, off flavors, etc., have been attributed to unsatisfactory powders.[3,9] Costilow *et al.*[9] obtained the most viscosity in cultured buttermilk made from reconstituted milk by pasteurizing at 180 F for 30 min; 180 to 190 F for 30 min also has been recommended.[3] Experiments [3,8] indicate that it is necessary to develop more acid in cultured buttermilk made from reconstituted milk than in that made from fresh milk; a titratable acidity in the range 0.90 to 1.0% appears most desirable.

Acetylmethylcarbinol plus biacetyl determinations conducted by Schock and Breazeale [3] indicated that the marked increase in these compounds in cultured buttermilk made from reconstituted skim milk occurred at a higher titratable acidity than when fresh skim milk was used and that the values never approached the values obtained with fresh milk, even when the solids content was somewhat higher in the reconstituted milk. Half fresh and half reconstituted milk gave carbinol plus biacetyl contents intermediate between those for fresh

and reconstituted milk. Both citric acid and sodium citrate were very effective in increasing the amounts of carbinol plus biacetyl in cultured buttermilk made from reconstituted skim milk; usually the compounds gave larger increases in carbinol plus biacetyl on holding the product cold for 48 hr after incubation than when fresh skim milk was used. Addition of citric acid or sodium citrate also increased the content of carbon dioxide. The carbon dioxide gave ripened buttermilk a desirable flavor which tended to mask the powder flavor initially evident. This effect of the gas could be demonstrated by placing buttermilk in a suction flask and removing the dissolved gases with a vacuum pump. The degassed buttermilk had a flat flavor, and the powder flavor became noticeable. A powder flavor was not detected in cultured buttermilk made from 10% reconstituted skim milk plus 2% fat and 0.15% sodium citrate. Since appreciable increases in carbinol plus biacetyl occurred when cultured buttermilk made from reconstituted skim milk was held cold for 24 to 48 hr, it was recommended that products reach the consumer when 1 to 2 days old.

After cultured buttermilk has attained the desired acidity, the curd is broken to a smooth consistency and cooled to 40 F or lower as rapidly as possible and with minimum agitation. When cooled, it is held for 3 to 4 hr without agitation before bottling. Breaking the curd and bottling immediately without cooling may give a porous product showing whey separation. Incorporation of air, storage above 40 F, and disturbance during the incubation also may cause whey separation.[3]

Cultured Sour Cream. A product which is essentially like cultured buttermilk except that it is made from cream is sold under such names as cultured sour cream, devonshire cream, etc. The product is used to embellish or enrich such foods as soups, salads, fruits, vegetables, meats, fish, and cheeses. In cookery, sour cream may be used in place of cultured buttermilk or sweet cream.[10]

In the manufacture of sour cream, raw cream containing about 18% butterfat is pasteurized, homogenized, and cooled to 72 F. Ripening is accomplished by use of a lactic culture. A small amount of rennet may be added at the time of inoculating with lactic culture to aid in maintaining a uniform viscosity from batch to batch. After ripening to the desired acidity, the cream is cooled and held cold until packaged. Guthrie [10] stated that the selection of a suitable culture is important for production of sour cream with a desirable body. Cooling cream after ripening and prior to agitation tends to eliminate free moisture and a grainy condition. A metallic flavor is evident in some

lots of sour cream; the defect may be due to contamination with copper and/or iron derived from equipment coming in contact with the product.

Bulgarian Milk. In some instances milk cultured with *L. bulgaricus* is desired for special purposes. *L. bulgaricus* is more difficult to propagate in pasteurized milk than lactic cultures because it must be grown at 90 to 110 F, and these temperatures favor growth of organisms resisting pasteurization exposures. Pasteurization on 2 successive days at 180 to 200 F for 30 min commonly aids in avoiding undesirable fermentations, as does the selection of milk with a low bacterial count. Sterilized milk can be employed and is satisfactory except for the heated flavor and the color produced by the high exposures necessary. Cultures of *L. bulgaricus* used for inoculating large volumes of milk should be carried in sterilized milk to avoid contamination. Milk fermented by *L. bulgaricus* does not have a flavor that is pleasing to most persons, and the flavor can be improved by adding lactic culture to it. Attempts to grow *L. bulgaricus* and the culture organisms together usually have not been successful because of the difficulty in maintaining a satisfactory balance between the various species.

Cultures of *L. bulgaricus* have been employed to prevent wheying off of buttermilk [11] obtained when sour cream is pasteurized and churned. A ropy strain of *L. bulgaricus* is used, and milk fermented by it is added to the buttermilk and thoroughly distributed. The heavy body of milk fermented by the ropy *L. bulgaricus* tends to prevent settling of the casein in the buttermilk.

Bulgarian milk has not given satisfactory results in many of the attempts made to control the intestinal flora through the consumption of milk cultures of it, and milk fermented by *L. acidophilus*, a normal intestinal form, is used more widely.

Acidophilus Milk. Milk fermented by *L. acidophilus* is marketed under the name of acidophilus milk.[12,13] This organism also is carried in pasteurized milk with considerable difficulty; it grows comparatively slowly and the temperatures used, which ordinarily are from 95 to 100 F, are favorable for the species resisting pasteurization. *L. acidophilus* is grown most commonly in sterile milk, either in flasks or in special tanks that permit heating the milk under pressure. Controlled methods of producing the original milk make it possible to use low sterilization exposures and thus obtain a product with less heated flavor and less change in color. Cultures used for inoculation must be carried under controlled conditions to insure purity, since a few

foreign organisms may quickly dominate the fermentation. Micrococci are common contaminating species although spore formers also are encountered. *L. acidophilus* sometimes is grown in whey instead of milk.

In general, it appears that large doses of *L. acidophilus* organisms taken regularly over a period of days or weeks, especially with milk or certain carbohydrates, tend to establish *L. acidophilus* as a predominant intestinal organism, frequently to the exclusion of certain other types. Lactose appears to be a particularly satisfactory carbohydrate, presumably because of its relatively slow absorption from the intestinal tract; for consumption it can be dissolved in water or milk. Results of various investigations indicate that *L. acidophilus* colonizes in the intestinal tract much more readily than *L. bulgaricus*. Some strains apparently colonize more successfully than others.

In the use of acidophilus milk, viability of the organisms is of primary importance. Apparently, the holding temperatures should not be too low. Kulp [14] found that in order to insure satisfactory viability for 2 days to 1 week commercial acidophilus milk of good quality may be stored at 41 F, provided the acidity at bottling is not above 0.65%. When a satisfactory viability for more than 1 week is desired, the following points are important: (1) Numbers of foreign bacteria must be negligible; (2) there must be rigid precautions against excessive acidity; (3) storage temperature should range between 53.6 and 60.8 F, the optimum viability temperature for *L. acidophilus* in milk being 60.8 F with an initial acidity of 0.65%.

Kopeloff *et al.*[15] reported that, in milk at the same titratable acidity, viability of rough and smooth strains of *L. acidophilus* differed at 39.2 F as well as 68 F. The rough strain lost its viability rapidly at 39.2 to 48.2 F, as compared with storage at 68 or 77 to 86 F. With an initial titratable acidity of 0.6 to 1.0%, the original count diminished approximately 90% in 3 days in the ice box and between 20 and 75% at room temperature. The smooth strain lost its viability less rapidly than the rough strain but storage in the ice box was more detrimental than storage at room temperature.

For persons who dislike the flavor of ordinary acidophilus milk, Myers [16] suggested the use of unfermented acidophilus milk, which consists of highly pasteurized sweet milk with many *L. acidophilus* cells suspended in it. The organism is grown in a sterilized medium and the cells then separated and added to the milk. Unfermented acidophilus milk is handled in the same manner as market milk and when stored at 35.6 to 41 F keeps sweet as long as 7 days. Souring

is rapid at room temperature, 50 F being the maximum temperature at which the milk can be stored satisfactorily. With the product, successful implantation of *L. acidophilus* in the intestinal tract was obtained in rats and humans. Morey [17] noted that a concentrate of *L. acidophilus* added to sweet milk results in a product containing over 500 million organisms per ml. For those who like buttermilk, a concentrate of *L. acidophilus* can be added to cultured buttermilk.

L. acidophilus also has been included in sherbets, candies, jellies, and various other materials; maintenance of viability of the organisms is a problem with all of these products. Prouty and Bendixen [18] found it possible to prepare sherbets in which large numbers of *L. acidophilus* remained viable after 5 to 7 days at temperatures below 1.4 F when more resistant strains with a high initial count were used and titratable acidity did not become excessive, preferably not above 1%.

Kefir. Kefir [19] is a type of fermented milk in which there is development of both acid and gas. It apparently originated among the people living in the Caucasus Mountains and surrounding territory and is made from milk of various species of animals. The fermentation can be started through use of kefir grains, which are convoluted masses made up of milk constituents and many micro-organisms. The grains are transferred from fermented milk to lots of fresh milk, or they can be dried and preserved and then soaked when needed. To return dry kefir grains to active condition, Burkey [19] suggested that they be soaked 2 to 3 hr in tepid water and then for a few hours in 1% sodium bicarbonate. In the dry condition the grains are yellow; during active growth they are almost white. When added to milk the grains settle to the bottom, but in the fermentation process they may be carried to the top by the gas developed. The grains increase in size during continued use and, occasionally, break up so that an abundant supply is provided. Under primitive conditions the fermentation commonly is carried out in leather bags and, as the kefir is used, fresh milk is added. When kefir is to be removed, the portion wanted may be tied off from the remainder so as to prevent escape of large quantities of gas when the bag is opened.

Figure 35. Actively growing kefir grain.

Various organisms have been isolated from kefir and it is probable that the species present are not constant. Streptococci and lactobacilli are involved in the acid development, and the gas often is produced by lactose-fermenting yeasts. Alcohol in variable quantities is formed

along with the gas; it is small in amount in the freshly made product but may increase considerably with age.

A product showing an acid and gassy fermentation can be made without kefir grains. The acid fermentation commonly is carried out first and gas production then is brought about through action of a yeast; if sealed containers are employed, much of the carbon dioxide can be retained. The general procedure is as follows: [20] Milk is fermented with a lactic culture in the usual way. It is put into bottles, about 4% sucrose added and dissolved, and then a small amount of bakers' or brewers' yeast, suspended in water, is inoculated. The bottles are stoppered tightly, the corks wired in if they are not of the picnic type, and the material held at about 70 F. Sucrose is added because the yeast does not ferment lactose and for many persons sugar improves the flavor of the product. When the fermentation is sufficiently advanced, the milk should be cooled to check growth of the organisms. Cooled bottles also can be handled with less danger of breakage than uncooled ones because of the greater solubility of the gas at lower temperatures. It is not unusual for a bottle to be shattered by the gas pressure. A product showing acid and gas also can be made from whey.[20] There is less danger of bottle breakage with whey than with milk. With milk, the casein frequently is in masses, whereas with whey, casein is present only in negligible amounts.

Kumiss. Kumiss, which is a product showing an acid and gassy fermentation, is made in certain parts of eastern Europe and western and central Asia. It commonly is made from mare's milk by inoculating with milk that has been fermented. When it is necessary to establish the fermentation, some fermenting or decaying material, such as flesh, tendon, or vegetable matter, can be added to the milk. The flora of kumiss apparently varies just as does that of kefir.

Yogurt. Yogurt is a fermented milk which differs from other fermented milks in that it is consumed as a custard rather than as a liquid. The yogurt-type of milk is made and used chiefly by the peoples of southeastern Europe, southern Asia, northern Africa, and particularly in the countries bordering on the eastern end of the Mediterranean Sea. Included in it are the yogurt of Turkey, the kisselo mleko of the Balkans, the mazun of Armenia, the gioddu of Sardinia, the dadhi of India, and the leben of Egypt.[21]

Milk used for yogurt commonly is boiled before it is inoculated, and the boiling may be continued until the milk is greatly concentrated. Under more modern conditions, concentrated or dry milk

solids may be added to milk to raise the solids content and the mixture homogenized and pasteurized before inoculation. Satisfactory yogurt cultures contain a *Lactobacillus* (probably *L. bulgaricus*) and *S. thermophilus*.[22,23] Rosell [24] considered three species desirable. The organisms in yogurt grow more rapidly and bring about more extensive changes in milk when grown together than when grown separately.[22,25] The more rapid acid production by the organisms in combination presumably is due to stimulation of the cocci by the rods. Pette and Lolkema [26] stimulated acid production of *S. thermophilus* by addition of a bacteria-free filtrate of *L. bulgaricus*.

Milk inoculated with yogurt culture is incubated at 104 to 115 F and coagulation is very rapid. The optimum growth temperature is somewhat lower for *S. thermophilus* than for *L. bulgaricus* so that the incubation temperature affects the proportion of the organisms.[27] The optimum proportion between rods and cocci in ripe yogurt is 1 to 1. In addition to temperature, the proportion is influenced by the incubation time. The cocci are stimulated in the early stages of growth by the lactobacilli and predominate when the acidity of the culture is low. The lactobacilli increase mainly when the acidity is high.

Figure 36. Bacteria in yogurt.

In order to determine the organism responsible for the flavor of yogurt, Pette and Lolkema [28] distilled 1-liter quantities of pure cultures of *S. thermophilus* and *L. bulgaricus* and added 2,4-dinitrophenylhydrazine to the distillates. Four cultures of *S. thermophilus* yielded 6 to 11 mg hydrazone per liter whereas 4 cultures of *L. bulgaricus* gave 16 to 50 mg per liter. The amount of hydrazone in distillates from a yogurt culture increased with acidity. No hydrazone was formed in a distillate from a culture having 0.29% titratable acidity, but 10, 20, 48, and 60 mg per liter were formed at acidities of 0.52, 0.67, 0.81, and 0.84%, respectively. The amount of derivative did not increase beyond an acidity of 0.84%. Yogurt with a titratable acidity of 0.84 to 0.90% was most satisfactory from the standpoint of flavor. Acetaldehyde was present in distillates from yogurt but was not considered to be an aroma substance.

Two cases of slow souring of yogurt were investigated by Pette and Kooy; [29] the defect was due to bacteriophage active against *S. thermophilus*, and two phages were isolated. A resistant strain of *S. therm-*

ophilus was obtained by continuing cultivation of a subculture from an infected culture until curdling had taken place.

Results of Seneca *et al.*[30] indicated that yogurt possesses both bacteriostatic and bactericidal properties against a wide range of pathogenic and nonpathogenic bacteria and protozoa and that these properties are related directly to the lactic acid content of the preparation. The antibiotic factor was diffusible and filterable, relatively heat stable, and lost its activity on neutralization.

Yogurt or yogurt culture has been used in the manufacture of other dairy products. Procedures have been outlined for the manufacture of yogurt cream[31] and fresh fruit yogurt sherbet.[32]

Holding Fermented Milk. One of the serious problems with fermented milk is keeping it in good condition after the fermentation is complete. With the large numbers of organisms present, growth is likely to continue after the desired stage of development is reached and the product then deteriorates. With buttermilk, low temperatures provide the most satisfactory method of holding and, if the product is to be kept for some time, it should be held at 40 F or lower. The buttermilk offered for sale often is of poor quality; frequently, the product has a yeasty and moldy flavor and is quite unsatisfactory to anyone familiar with a high-quality product. While the acid developed in buttermilk may prevent growth of many organisms, certain species can tolerate it, the yeasts and molds being especially important. Manufacture and handling of buttermilk under such conditions that yeasts and molds are not present is an aid in marketing a high-quality product, but the real solution of the holding of buttermilk is employment of storage temperatures that are so low growth of all organisms is delayed materially.

With acidophilus milk, holding temperatures should be used that favor retention of viability by *L. acidophilus*. When a fermented milk involves formation of gas as well as acid, holding under unsatisfactory conditions may result in bursting of the containers.

REFERENCES

1. *Milk Dealer,* **42** (6), 68 (1953).
2. *Milk Dealer,* **30** (6), 41 (1941).
3. *S. Dak. Agr. Expt. Sta. Bull.* 391 (1948).
4. *J. Dairy Sci.,* **37,** 22 (1954).
5. *J. Dairy Sci.,* **37,** 1128 (1954).
6. *Milk Dealer,* **33** (4), 32 (1944).
7. *J. Dairy Sci.,* **13,** 124 (1930).
8. *Ala. Agr. Expt. Sta. Bull.* 239 (1933).
9. *Milk Plant Monthly,* **38** (4), 36 (1949).

10. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 880 (1952).
11. *Ill. Agr. Expt. Sta. Cir.* 166 (1913).
12. *Neb. Agr. Expt. Sta. Bull.* 228 (1928).
13. *Ore. Agr. Expt. Sta. Tech. Bull.* 5 (1944).
14. *Am. J. Public Health,* **21,** 873 (1931).
15. *J. Bacteriol.,* **28,** 489 (1934).
16. *Am. J. Public Health,* **21,** 867 (1931).
17. *Milk Dealer,* **43** (4), 65 (1954).
18. *J. Dairy Sci.,* **15,** 413 (1932).
19. *U. S. Dept. Agr., Bur. Dairy Ind., Mimeo Inf.* 58, Nov. 1947.
20. *Ia. Agr. Expt. Sta. Cir.* 54 (1918).
21. *U. S. Dept. Agr., Bur. Dairy Ind., Mimeo Inf.* 2, May 1947.
22. *Food Eng.,* **24** (3), 59 (1952).
23. *Australian J. Dairy Technol.,* **6,** 123 (1951).
24. *Can. Public Health J.,* **24,** 344 (1933).
25. *Neth. Milk Dairy J.,* **4,** 197 (1950).
26. *Neth. Milk Dairy J.,* **4,** 209 (1950).
27. *Neth. Milk Dairy J.,* **5,** 14 (1951).
28. *Neth. Milk Dairy J.,* **4,** 261 (1950).
29. *Neth. Milk Dairy J.,* **6,** 233 (1952).
30. *Am. Practitioner and Dig. Treatment,* **1,** 1252 (1950).
31. *Milk Plant Monthly,* **40** (4), 54 (1951).
32. *Ice Cream Rev.,* **34** (9), 41 (1951).

Bacteriology of Butter

18

The bacteriology of butter differs in various respects from the bacteriology of milk. Because of its composition, butter is less satisfactory than milk for growth of most micro-organisms. Lactose, which is attacked readily by many species, is present in small amounts, whereas approximately 80% of the product consists of the relatively resistant fat. The moisture content is low and, except with unsalted butter, commonly contains a comparatively high concentration of sodium chloride. Moreover, the moisture is in droplets that are surrounded by fat so that water is not the continuous phase, as it is in milk. Most of the organisms are in the water and, since there are many more droplets than there are organisms, a large proportion of the droplets are sterile and must remain so because bacteria cannot migrate through the fat. The holding period with butter often is comparatively long because of the time required for it to pass through the usual channels to the consumer. Although butter in storage is held at 0 F or lower, in stores and homes the temperatures often are such that many bacteria can multiply, provided they tolerate the other conditions imposed by the butter.

Lactic culture is employed much less in the butter industry than formerly but still is used in some salted butter and in much of the high-flavored, unsalted butter intended for table consumption. Ac-

cordingly, butter sometimes contains considerable numbers of bacteria intentionally added.

From the standpoint of the microbiology of butter there is a striking difference between the salted and the unsalted products. The salt definitely inhibits growth of various species of organisms, and may even kill some of them, so that deterioration through growth of organisms presents more of a problem with unsalted than with salted butter. On the other hand, certain undesirable chemical changes, favored by the salt, occur more readily in the salted product.

Most butter still is made by agitating individual lots of cream in churns, but continuous methods of butter manufacture are coming into use. Although the products made with these methods present the same general microbiological problems as churned butter, with at least some of the methods control of contamination is simplified and the fine moisture distribution in the butter tends to limit bacterial growth so that the butter is comparatively resistant to bacterial spoilage. Methods of making sterile butter, using high processing temperatures, rapid chilling, and aseptic canning, also are being investigated.

Determination of Numbers of Bacteria. The numbers of bacteria in butter can be determined by the plate method, the microscopic method, or an adaptation of the Burri technic. Because of its physical condition, the sampling of lots of butter presents difficulties not encountered with milk. Standard methods [1] for sampling butter in churns, in packages, and in prints, and for the care of samples have been established.

Plate Count. Plating butter is more difficult than plating milk because of the solidification at ordinary temperatures and the tendency of the fat to form large globules in the medium, even when melted. Water blanks, pipettes, and plates should be warmed to a temperature above the melting point of the butter; this can be done conveniently by allowing them to stand in a 113 F (45 C) incubator, or the blanks can be placed in warm water. Portions for plating can be weighed, using precautions against contamination, but it is more convenient to place the sample jar in a water bath at 104 to 113 F (40 to 45 C) until the butter can be shaken to a thin, creamy consistency, and then obtain the portions required with a warmed pipette. The outside of the pipette is kept relatively free of butter and the pipette is emptied as completely as possible; when butter is being added to a water blank the pipette is rinsed by drawing the warm water into it. After the butter is in the first water blank, dilutions are made as with milk. Because the fat quickly comes to the top

of a blank on standing, it is necessary to work rapidly and shake the blank just before a portion is removed. Excessive shaking, especially if the butter is not thoroughly melted, may cause the fat to form clumps and thus interfere with its distribution. The dilutions used depend on the number of organisms expected from the history of the sample.

Any agar that is satisfactory for plate counts on milk can be used for butter; beef infusion and tryptone glucose extract (milk) agars have been employed extensively. By adding skim milk and/or fat, differential counts also can be made. Incubation of the plates 3 to 5 days at 70 to 77 F (21.1 to 25 C) is advisable. In some cases additional incubation of 2 days at 95 F (35 C) is used.

Microscopic Count. Various procedures have been used in making microscopic counts on butter. Hammer and Nelson [2] developed a method in which 10 ml of butter that has been warmed to 113 F (45 C) is centrifuged in a separatory funnel and the serum drawn off and examined, essentially as in the microscopic count on milk. A 0.01-ml portion of the thoroughly mixed serum is spread over an area of 1 to 8, or more, sq cm; if the area is larger than 1 sq cm, distilled water, or milk free of stainable cells, is used in the spreading. Results are calculated to the basis of 1 ml of serum, and then to the basis of 1 ml of butter by dividing by 9, since there are only insignificant numbers of organisms left in the fat and the volume of the serum is approximately one-ninth that of the original butter. Because all commercial butter is made from pasteurized cream and many of the dead bacteria still are stainable, the microscopic picture must be interpreted properly. Often the living organisms stain more intensely than the dead ones.

In the examination of defective butter, general observations on the numbers and morphologic types of organisms present often are advisable. Hussong [3] suggested a procedure that can be applied quickly with very little equipment. A small piece of butter is placed on a slide and melted. A few drops of acetone are added and mixed with the butter by moving the slide or stirring with a flamed needle. The acetone-fat-water mixture is poured off gently, leaving the curd and other materials on the slide. A second treatment with acetone completes removal of the fat. After evaporating the remaining acetone, the slide is stained and examined. Xylene sometimes has been used instead of acetone.

Burri Technic. Long and Hammer [1] adapted the Burri smear culture technic to the examination of butter. The butter is placed in a

sterile petri dish and brought to about 69.8 F (21 C), at which temperature small amounts can be picked easily. Under a binocular giving about a 6× magnification, small amounts (approximately 1/20,000 g each is satisfactory) are picked with a flamed platinum needle; the binocular aids in keeping the portions uniform in size and in actually picking each portion rather than scraping it from a relatively large area. Each portion is spread on the surface of a dry agar slope. Ordinarily, 25 portions are picked, but larger or smaller numbers may be used to give more or less detail in the results. The slopes are incubated at 69.8 F for 4 or 5 days and counted. The amount of butter actually being picked can be estimated by determining the collective weight of a number of portions. The method gives results lower than the plate method, presumably because clumps of bacteria are not broken as they would be in a water blank; also, there may be overcrowding on slopes with high-count samples. The main advantage of the procedure is in studying the distribution of organisms from point to point in a sample.

Determination of Numbers of Yeasts and Molds. Yeast and mold counts on butter are made to obtain information on the efficiency of plant operations from the sanitary standpoint. With satisfactory plant methods, yeast and mold counts are very low; with unsatisfactory methods, they often are high. Usually, yeast and mold counts are made by the plate method, but a microscopic colony procedure also has been suggested.[5]

Plate Count. Yeast and mold counts on butter are made with a standard procedure [1] that consists of plating on potato dextrose agar acidified to *p*H 3.5 ± 0.1 with sterile tartaric or lactic acid to prevent development of bacterial colonies; the medium is convenient, inexpensive, and very satisfactory. When the yeast and mold counts are low, plates must be poured with 1-ml quantities of butter. This requires properly warmed equipment and rapid manipulation, if the fat is to be well distributed through the agar; without proper distribution some yeasts and molds may be completely enclosed in fat and their growth delayed or entirely prevented. Rapid cooling of freshly poured plates on a chilled surface helps keep the fat well distributed.

Before the establishment of standard methods for yeast and mold counts on butter, various media and acidification procedures were employed. Hood and White [6] noted the variation in *p*H values of the media and pointed out that some of them permitted growth of bacteria. They found that all bacterial growth was inhibited by a *p*H of 3.5. Wort, malt, whey, and potato were equally suitable as a nutrient

base for yeast and mold counts when the *p*H was 3.5. Later studies [7, 8] also show the importance of a *p*H of 3.5. It does not significantly affect development of yeasts and molds, and at higher values bacteria often grow.

Various incubation conditions have been employed for the plates used in determining yeasts and molds in butter. In the studies of

Figure 37. Agar plate poured with butter, showing yeast and mold colonies; also fat globules.

White and Hood [9] the average yeast and mold count on all samples, and counts on the majority of the individual samples, were slightly higher at 77 F (25 C) than at 86 F (30 C) with an incubation of 48 hr or 5 days, although the differences were not of practical significance. At 98.6 F (37 C) the counts nearly always were appreciably lower than at 77 or 86 F. Plates now commonly are incubated 5 days at 69.8 or 77 F (21 or 25 C). If many molds that tend to overgrow the plates are present, it may be necessary to count the plates earlier.

Commonly, the yeast and mold colonies on a plate are counted separately although the two types of organisms are equally suggestive of contamination; in some cases differentiation by simple colony appearance may be inaccurate. With high total counts, yeasts usually are the more numerous. Holding the plates at a temperature some-

what above the melting point of the fat for a short time just before counting so changes the appearance of the fat globules that they are not confused with yeast colonies. Apparently, there are many instances of one species interfering with, or modifying, the growth of another so that an irregular distribution of the colonies on a plate often is noted.

Standards for Yeast and Mold Counts. Various standards have been suggested for yeast and mold counts on butter, such as <5 or <10 per ml; in some cases limits have been set for excellent, good, and fair counts, etc. However, the objective should be to have all samples from a plant show <1 per ml. Until this goal is reached, there is opportunity for improvement.

Sources of Organisms in Fresh Butter. The organisms in fresh butter come from various sources, the relative importance of the sources depending on the conditions of production. In a well-operated plant, the cream that has been prepared for churning contributes most of the organisms; with insanitary methods, many organisms come from other sources, especially the equipment.

Cream. Sweet cream used in the manufacture of butter varies widely in its microbiological condition. Although some of it is skimmed in plants and may contain comparatively few organisms, some of it is separated on farms and often is held there at temperatures that permit growth. Normally, bacteria greatly outnumber the yeasts and molds, but the latter groups regularly are present.

Sour cream used for butter inevitably contains large numbers of organisms since many are required to produce the acid present. It regularly contains hundreds of millions of bacteria per ml. With moderate acidities *S. lactis* is the predominant species, and with high acidities both *S. lactis* and lactobacilli are prominent. Various other bacteria also are present, the number and species depending primarily on the contamination of the cream and conditions of holding. Since yeasts and molds regularly are present in sweet cream, they also are found in sour cream in which they may have grown extensively.

In the pasteurization of cream intended for butter, exposures higher than those used for market milk often are employed. With HTST heating 195 F or higher may be used, and with LTLT heating 165 F may be reached. Most of the organisms in the cream are killed easily by heat so that pasteurization efficiencies are high. Defects that develop in butter through activities of organisms normally are due to contamination of the pasteurized cream or the butter, rather than to organisms surviving the heat treatment.

Culture. When culture is used in the cream churned, most of the culture organisms remain in the buttermilk, but some of them are present in the butter. If culture is worked into the butter, the organisms largely remain in the product.

General Equipment. Unless properly cared for, piping, pumps, coolers, vats, etc., are sources of contamination with pasteurized cream intended for butter, just as with milk. Cream storage vats with breaks in the lining may cause serious contamination; often such breaks are so small they are difficult to detect but contaminating material still may be forced through them, especially when the vat is full.

Special Equipment. Churns constitute the characteristic equipment of butter plants and, without proper care, they are important sources of the organisms in butter. Metal churn barrels have an advantage from the sanitary standpoint, but many barrels still are made of wood. The surface of the wood is more or less irregular, the irregularities increase as the wood takes up water, and the wood may crack and sliver so that removal of organisms and milk solids is difficult. The heavy loads that churns must carry and the strain thrown on them during the working of butter tend to open joints in the wood, loosen bolts, etc., and thus further increase the difficulty. When churns were improved mechanically by building into them the equipment for working butter, they became more of a problem from the sanitary standpoint. A churn can be studied for the numbers of organisms contained by the rinse method or the agar disc method (see p. 130), but neither gives an adequate picture because of the many inaccessible points in a churn.

Various investigators have emphasized the importance of churns as a source of organisms in butter and also the difficulty of keeping them in a satisfactory microbiological condition. Libbert [10] isolated 57 yeast and mold cultures from churns, and all except 3 of the yeast cultures grew on a medium containing water, agar, and ground fir wood. By dismantling 2 churns which had been in service for a number of years and culturing samples of scrapings, wood, etc., Macy *et al.*[11] demonstrated that numerous contamination foci exist in a churn. Of 230 samples from a churn receiving ordinary treatment, 192 yielded molds; of 73 samples from a churn receiving careful treatment, only 22 yielded molds and, in general, the contamination was light. Yeasts were present in the majority of the samples and bacteria always were present, sometimes in large numbers. With the agar disc method Olson and Hammer [12] found that 27 churns in commercial use in 24 plants varied widely in microbiological condition, some of

them containing comparatively small numbers of organisms and others containing excessive numbers. Bacteria regularly were much more numerous than yeasts or molds, and molds usually were somewhat more numerous than yeasts. When the bacterial counts were low, comparatively few species were present and *Bacillus* and *Micrococcus* organisms predominated; when the counts were high, many species commonly were present. In studies on *Pseudomonas putrefaciens*, Long and Hammer [13] isolated it and also other species, including yeasts and molds, from various points in churns which were reached by loosening bolts, dismantling parts, etc.

The first butter of the day from a churn sometimes shows a higher content of bacteria, yeasts, and molds than the second churning, even when cream from the same lot is used. This is due to elimination of many of the organisms from a contaminated churn during the period of use and is convincing evidence of the importance of churn sanitation.

Churn Treatment. After use a churn should be thoroughly cleaned and then sanitized with hot water. A general procedure for the care of a churn is as follows: Immediately after use the milk solids are rinsed from the churn by adding water at 100 to 120 F, revolving for several minutes, and draining. The churn next is filled one-third to one-half full of water at 170 to 180 F, washing reagent added, the churn revolved in high gear for 15 min, and drained. The churn then is filled one-half full of water at 200 F or higher, revolved in high gear for not less than 45 min, and drained thoroughly. Heat from the water penetrates the wood very slowly. In some plants the hot churn is dried by holding with the doors up for a few minutes to permit the escape of moisture, again draining, and again holding with the doors up; when dry, the churn is left with the doors two-thirds up and covered with a clean wire screen or muslin on a frame. Before use the churn is rinsed with cold water containing a bactericide. In other plants, the hot churn is partly filled with cold water containing a bactericide, revolved for a few minutes, and then held until needed with the water in it.

When a churn is contaminated to the point where it is causing spoilage in the butter made in it, elimination of the contamination often requires repeated and prolonged treatment with very hot water. Organisms at deeper points in the churn are difficult to reach with either heat or a bactericide, and heat seems to be the more effective. Under these conditions, some plants leave the churn over night with

hot water in it, although such severe treatment often ruins paint on the churn.

In some instances plant operators fail to treat a churn properly with moist heat because of the effect on the wood. However, adequate destruction of the organisms left in a churn after use is essential in the modern manufacture of butter. Quality of the wood is an important factor in the life of a churn. In some instances one or more pieces of wood in a churn deteriorate very rapidly, regardless of the churn treatment. Many churns made from good-quality wood have been in use over extended periods with adequate heat treatment.

Wash Water. Wash water is a source of organisms in the butter on which it is used. With a satisfactory supply the numbers and types of organisms from this source are of no significance, whereas with a contaminated supply organisms added to the butter may be the cause of serious deterioration. Many outbreaks of spoilage in butter have been traced to contaminated water supplies (see Chapter 20).

Air. Air contamination is more important in a butter plant than in other dairy plants because butter often is exposed rather extensively during the packing, it frequently is held for relatively long periods at temperatures permitting growth of organisms, and development of molds on butter is a serious defect.

Olson and Hammer [14] studied the numbers of bacteria, yeasts, and molds falling from the air by exposing agar plates once a week for approximately 1 yr at 6 locations, 3 in dairy plants, 1 in a laboratory, and 2 out-of-doors. In general, the 3 groups of organisms were falling rather constantly, bacteria being the most numerous and yeasts the least numerous. The numbers of micro-organisms falling indoors did not differ greatly from the numbers falling out-of-doors, and there was no distinct seasonal variation. A muslin cover reduced the number of organisms falling on a surface.

Butter Color. Jamieson and Chen [15] made plate counts on butter color and found that, as it comes from the manufacturer, it is practically free of micro-organisms. It may be contaminated in the plants by holding in opened containers, from the lip over which it is poured, or from unwashed measuring glasses. There was no conclusive evidence of growth of organisms in the product. Many of the bacteria and some of the yeasts were types that are detrimental to butter quality.

Plant Employees. Persons making and handling butter may add organisms to the cream used and to the finished butter. They come from the hands, mouth, nasal passages, and clothing. Molds carried

to the finished butter as a result of handling raw cream, cans, etc., may be especially serious from the standpoint of mold growth on butter.

Packaging Materials. As packaging materials come from the manufacturers, they normally are in a satisfactory microbiological condition. With proper care they often are used without any plant treatment. Apparently, treatment is most common with box liners. Some butter purchasers may require that liners be immersed in hot salt water, or a chemical solution, to help avoid molding of the product.

Comparative Numbers of Organisms in Cream and Butter. When cream is churned the great majority of the organisms originally in the cream are present in the buttermilk so that fresh butter contains comparatively few. Grimes [16] reported that butter made from cream in which culture was used retained about 0.5 to 2.0% as many bacteria per milliliter as were present in the cream; with sweet cream containing no culture, the values were about 5 to 30%. Macy *et al.*[17] found that the bacterial count per milliliter of butter commonly was lower than that of the cream in the churn. The yeast and mold counts often were higher on the butter; this was attributed to contamination from remote parts of the churn and, with molds, to the breaking up of aggregates of conidia.

In controlled experiments Parfitt [18] inoculated pure cultures of yeasts or bacteria into sterile cream and then churned it in sterile jars. The numbers of organisms per milliliter in the unsalted butter always were smaller than the numbers in the cream. The wash water removed considerable numbers of organisms. With the yeasts the counts on the butter were 1.8 to 27.3% (average 13.4) of the counts on the cream; with the bacteria the percentages ranged from 0.3 to 10.5 (average 3.9). Elliker [19] found that during the churning of cream to which culture had been added, bacteria tended to go into the buttermilk while many more were removed by the wash water. Percentages of the total bacteria in the different fractions were butter 0.75; buttermilk 91.57; and wash water 7.68.

Elimination of large numbers of organisms in the buttermilk is a desirable thing from the standpoint of the deterioration of butter since it results in contamination of a smaller percentage of moisture droplets than otherwise would be the case.

Numbers of Organisms in Fresh Butter. The bacterial content of fresh butter is extremely variable and depends primarily on the method of manufacture. Although sweet cream contains fewer organisms than sour cream, pasteurization largely eliminates the original

difference, and there is no regular variation between the bacterial counts on sweet-cream and sour-cream butter. When culture is used in cream, either with or without actual ripening, the numbers of bacteria in the cream and in the butter made from it are increased. Ordinarily, use of culture overshadows extensive contamination from equipment as far as numbers of bacteria are concerned, but the species from the two sources differ strikingly. The bacterial content of fresh butter varies from a few thousand per ml, in the case of a product properly made from thoroughly pasteurized cream without culture, to several million when culture is employed and/or manufacturing methods are inadequate.

The numbers of yeasts and molds in butter also vary widely. Some samples may fail to show any of these organisms in 1 ml; counts on others run into the thousands.

Factors Limiting Growth of Organisms in Butter. A number of factors definitely limit growth of organisms in butter. Apparently, certain of them vary in their effects on different species.

Physical Condition. Various investigators have shown that the water in butter is divided into enormous numbers of tiny droplets, roughly from 10 to 18 billion per g. This distribution is of great significance from the standpoint of growth of organisms in butter. Rahn and Boysen [20] noted that a portion of the moisture carried from the cream into the butter becomes sterile through division into very tiny droplets and that the amount of moisture from which bacteria are excluded depends on two factors, number of bacteria in the cream at time of churning and degree of dispersion of the moisture in the butter.

Hammer and Hussong [21] found that multiplication of organisms in unsalted butter was more rapid when the serum was separated than when the butter was left in normal physical condition. This indicates that, with the serum separated, the nutrients contained are more readily available to the organisms present. With salted butter, separation of the serum increased the rate of destruction of organisms.

Using an adaptation of the Burri technic, Long and Hammer [4] examined very small portions of butter for their bacterial contents and noted an irregular distribution which often was extreme. This was considered evidence that bacterial growth in butter is limited largely to certain focal points, probably infected water droplets.

Scharp [22] concluded that, as the working of commercial unsalted butter is increased, the number of organisms after any holding period decreases; this was attributed to finer dispersion of the water. As judged by rate of growth, production of defects, production of acid

by culture organisms, and liberation of fatty acids by lipolytic bacteria, Long and Hammer [23] found that bacteria are more active in underworked than in thoroughly worked butter. Muller [24] developed a method for determining moisture distribution in butter and concluded that the microscopic picture is a satisfactory guide to the amount of working, the number of droplets greater than 30μ in diameter being inversely proportional to the time of working. There was a general relationship between droplet count and texture defects, and bacterial activity decreased as the moisture was more finely dispersed.

Reworking butter under certain conditions may have an effect on bacterial growth in it through the influence on moisture distribution. When butter is reworked in a churn to reduce the moisture content, it is cold enough so that water can be squeezed from it, but with butter printers having a reworking effect the product normally is warm enough to avoid significant loss of water from properly made butter. Practical experience indicates that butter from such a printer sometimes deteriorates rapidly, whereas unprinted butter from the same churning keeps satisfactorily. Apparently, redistribution of organisms and aggregation of water and the nutrients contained in it are responsible for the increased bacterial activity. Long and Hammer [25] found that reworking unsalted butter made from pasteurized cream inoculated with various organisms frequently increased the activity of organisms at 50 F, as evidenced by growth rates, production of defects, etc.

Collins and Hammer [26] studied migration of bacteria through unsalted butter held at temperatures favorable for growth and noted that it was not common. In the few instances in which migration occurred, poorly-worked butter was involved more often than well-worked butter. Bacteria sometimes migrated extensively along the wall of the container in which butter was packed, presumably because water collected there.

It is evident that the physical condition of butter is an important factor in restraining growth of bacteria in the product and preventing its deterioration. Organisms that can cause spoilage must be present in relatively large numbers if sufficient moisture droplets are to be infected to influence the product significantly. However, even with this restraining effect, large numbers of organisms sometimes are present in butter held under conditions favorable for growth. Counts in the hundreds of millions per ml have been noted with organisms well adapted to multiplication in butter.

In considering the moisture in butter, it should be recognized that

there are differences in the opportunities for the dispersion of moisture from different sources. Presumably, the opportunities are best with the moisture coming from the cream, somewhat less with moisture from the butter wash water, and least with moisture added late in the working of the butter to adjust the moisture content; water from the cream normally contains the most solids. Tuomainen [27] added fuchsin to cream and methylene blue to wash water and found that in the butter (unsalted) the smaller droplets usually were red and the larger ones blue.

It appears that moisture dispersion in butter is of much less significance in the growth of molds and yeasts than in the growth of bacteria.

Salt Content. Salt tends to retard growth of various bacteria, yeasts, and molds in butter and thus delays, or prevents, spoilage. Action of salt is influenced by concentration in the water droplets and by uniformity of distribution in them. Even with considerable salt in butter, the concentration in certain droplets may not be sufficient to inhibit bacterial growth. Instances have been reported in which a species relatively sensitive to salt has produced a conspicuous defect in commercial salted butter, presumably because of poor salt distribution. Tuomainen [27] found with color tests that there are salt-free water droplets in freshly worked, salted butter.

The influence of salt on growth of bacteria in butter varies greatly with the species present. In experimental churnings made from lots of cream inoculated with various organisms causing defects in butter, it commonly is noted that deterioration is prevented in some of the butter by salting, whereas when other organisms are involved deterioration is not prevented although it may be delayed. In general, the lactic culture species develop little, or not at all, in salted butter held at favorable growth temperatures, whereas in unsalted butter they develop extensively, as is evident from both plate and microscopic counts; [28] the most striking evidence of growth is the presence of long chains in the butter. It appears that some organisms decrease in numbers in salted butter under conditions which permit their growth in an unsalted product from the same churning.[21]

The presence of salt lowers the freezing point of butter so that organisms may grow at temperatures below 32 F. Rahn *et al.*[29] reported that some organisms multiplied in salted butter at 21.2 F. However, at such a temperature, growth is limited by both the temperature and the salt.

Studies on the distribution of salt in butter on a macro basis have

given information that is useful in composition control, etc., but, from the standpoint of bacterial action in butter, values on much smaller portions are desirable. Hoecker and Hammer [30] studied salt and moisture distribution in butter with samples that commonly were less than 1 mg, these being the smallest portions giving satisfactory analytical results. In some samples of normal butter the salt was uniformly distributed and in others it was not; with most samples there was a correlation between salt distribution and moisture incorporation. In general, as the working of butter continued, the salt became more uniformly distributed. When lots of pasteurized cream, each inoculated with an organism capable of producing a defect in butter, were churned and the butter held at 59.9 F, the butter with the salt well distributed usually did not develop defects, whereas the butter with the salt poorly distributed frequently did. Both thoroughly- and poorly-worked unsalted butter usually developed defects; they commonly appeared earlier in the poorly-worked product.

In considering the influence of salt on the organisms in butter, it should be recognized that the percentages of salt in various lots of butter differ widely so that there may be greater variation in salt content between two lots of salted butter than between certain lots of low-salted butter and unsalted butter. With this situation prevailing, information on the amount of salt present has a definite microbiological significance.

Temperature. Low temperatures constitute one of the important factors in the control of organisms in butter. Data of various investigators [31, 32, 33] indicate there is no significant growth of bacteria, yeasts, or molds below 32 F, and there may be a decrease in numbers. Above 32 F organisms may develop in butter, especially if it is unsalted. Commonly, growth is more rapid at somewhat higher temperatures than at 32 F. When unsalted butter is made from cream containing an organism capable of causing a defect and different portions are held at temperatures ranging from 32 to 70 F, the period required for the defect to develop decreases as the temperature increases. Bacterial growth in salted butter probably shows the same relationship.

Freezing butter, known to be susceptible to rapid deterioration, at commercial storage temperatures may not give a product that will keep when placed at temperatures favorable for deterioration. Turgasen [34] investigated the effect of extended holding of butter at −20 F on activity of organisms causing cheesiness; infected print butter stored for 6 months developed the defect when placed at 70 F.

Acidity. In butter, low *p*H values presumably limit, or prevent, growth of acid-sensitive organisms, just as in other products. With salted butter the *p*H commonly is kept relatively high, since acid favors certain types of chemical deterioration, and in the normal range for the product there appears to be no relationship between *p*H and growth of organisms.[35,36] The low *p*H of unsalted, ripened-cream butter definitely may restrain certain bacteria.

Air Supply. Butter held under conditions permitting growth of organisms often shows higher counts at, or near, the surface than in the interior, and certain microbiological defects of butter, especially the putrid defect, commonly are first evident at the surface. In general, these relationships are interpreted as effects of the air supply on growth of organisms. Extensive growth of organisms in butter ordinarily is followed by a decrease in numbers so that, with rather extended holding, the count at the surface may decrease to a point where it is lower than the count at the interior.[37]

General Considerations. Although there are various factors tending to restrain growth of bacteria in butter, increases in numbers and spoilage due to bacterial growth are common in the commercial product. For each restraining factor there are counterinfluences, and in some instances these outweigh the restraining effect. Hammer and Long [38] outlined the factors limiting growth and the counterinfluences as follows:

Factors Limiting Bacterial Growth in Butter and Counterinfluences

Factors Limiting Growth	Suggested Counterinfluences
Fine dispersion of water	High initial contamination resulting in many infected water droplets
	Printing butter under conditions tending to aggregate water droplets
	Action of salt in tending to aggregate water droplets
Addition of salt	Poor distribution of salt
	Relatively little salt added
	High initial contamination
	Presence of salt-tolerant species
	Adaptation of certain species to relatively high salt concentration
Low temperature	Presence of psychrophilic organisms that grow just above freezing point of water
	Exposure of butter for short periods (during handling, etc.) to temperatures allowing rapid growth
	Depression of freezing point of water in butter by salt
Use of lactic culture; acidity	*S. lactis* less salt-tolerant than many other species
	Inhibition of *S. lactis* but not psychrophilic species by holding temperatures
	Acid content of salted butter and some unsalted butter commonly too low to inhibit organisms
Air supply	Air incorporated in butter during manufacture sufficient for many species

Effect of Growth of Organisms in Butter. Some species of organisms grow extensively in butter without significantly influencing the flavor and, as a result, certain lots of butter with very high counts show no deterioration attributable to organisms. In general, such species also bring about changes in milk slowly. Other species produce changes in butter quickly when conditions for growth are favorable; commonly, changes in milk also are brought about rapidly.

Determination of Effect of an Organism on Butter. Study of the effect of an organism on butter presents difficulties not encountered with milk and cream which have the moisture in the continuous phase. Inoculation of an organism into butter is not satisfactory. The organism must be well distributed through the product, and this is accomplished best by inoculating it into cream and then churning. Ordinarily, experimental churnings involving from one to several pints of sterilized, or thoroughly pasteurized, cream are used. Precautions against contamination are advisable and should include use of sterile wash water, butter-working equipment, and containers. Some of the butter can be salted and some left unsalted, and portions can be packed for storage at different temperatures. Comparisons of the butter churned from inoculated cream with that churned from uninoculated cream should be made frequently since defects sometimes become conspicuous and then tend to change in character or become less pronounced.

SOURCES OF THE FLAVOR OF BUTTER

The general flavor of butter is the most important quality from the standpoint of market value of the product. It involves flavors from various sources and what the butter grader evaluates is a combination of flavors. These can be divided into two groups, (1) the desirable flavors, and (2) the undesirable flavors.

Desirable Flavors of Butter

The desirable flavors of butter come from two sources, the milk constituents and the products of bacterial growth or the added flavor concentrates.

Milk Constituents. Various milk constituents influence the flavor of butter. The fat is of most importance in this connection because of the high percentage in which it is present and its characteristic flavor. Butter churned from cream in which there has been no signifi-

cant development of organisms has a definite butter flavor that is pleasing and is desired in certain markets, but it lacks the high flavor, preferred by some critical consumers, which is present in butter made so that it contains products formed by certain bacteria or added as butter flavor concentrates. A high flavor in unsalted butter for table use is essential in many markets.

Products of Bacterial Growth. The cultures usually used in making butter are lactic cultures, but various special cultures also have been suggested. Because of its importance, action of lactic cultures in butter has been investigated extensively.[39]

Various procedures have been used in making butter with culture. It has been (1) added to the pasteurized cream at churning, (2) added to the pasteurized cream and the mixture held cold for some hours, (3) added to the pasteurized cream and the mixture ripened, or (4) added directly to the butter. Each procedure has its advantages and disadvantages. Addition of culture to cream carries flavor materials into the cream and butter. With holding or actual ripening, there may be production of flavor materials in the cream. Although for salted butter the *p*H should be kept relatively high, for unsalted butter no such precautions are necessary and full advantage can be taken of flavor production by the culture.

The chemical compounds involved in giving butter a desirable flavor through use of culture are volatile acids, biacetyl, and perhaps others; the same compounds give lactic cultures their characteristic flavor.

Volatile acids give butter a pleasing, slightly acid aroma; on the basis of the products formed in lactic cultures, acetic acid is of primary importance. Addition of acetic acid in the proper amount to sweet cream that is churned into butter without use of culture gives a pleasing flavor to the product, but it is not a typical culture flavor.

The studies of van Niel *et al.*[40] indicated that biacetyl is a flavor contributant of butter. In fine butter 0.0002 to 0.0004% biacetyl was found and, when these concentrations were added to butter neutral in odor, an unmistakable aroma appeared. Schmalfuss and Barthmeyer [41] noted the presence of biacetyl in various materials, including butter. Margarine to which biacetyl was added had the aroma of butter. In a number of samples of butter examined, the biacetyl content was correlated with the intensity of flavor.

Various analyses and many plant trials in which biacetyl has been added to butter indicate that the compound definitely contributes to the flavor of the product. The flavor concentrates that have been

developed for butter, and for products intended to have the flavor of butter, contain primarily biacetyl although other flavor materials also may be included. There is no close correlation between the biacetyl content and score of butter because off flavors that definitely lower the score cannot be covered by any normal amount of biacetyl and excessive amounts give butter an artificial flavor.

Pette [42] reported that fresh "aromatic" butter containing 1.0 mg. or more, biacetyl per kg scored substantially higher on aroma and flavor than "neutral" butter containing 0.5 mg. or less; after cold storage for 3.5 months, the aromatic butter still scored higher, but the difference was less. Holwerda and van der Gust [43] noted a direct correlation between the biacetyl content of unsalted, sour-cream butter and the grade, provided the butter containing over 10,000 yeasts or 100,000 acidophilic bacteria per ml was not considered. In the United States certain manufacturers now are marketing butter with a conspicuous biacetyl flavor. Although some consumers prefer butter that is lacking in biacetyl flavor, others desire such a flavor and it is essential in unsalted butter for table use.

Biacetyl has been found important in the food preferences of animals. Deuel and Movitt [44] noted that rats preferred a diet of mineralized, vitamin-fortified skim milk powder containing butter to one containing other fats, apparently because of flavor. Animals preferred a margarine or peanut oil diet containing 4 ppm biacetyl to an unflavored diet; they also preferred this level of biacetyl to a higher one. Commercial butter flavor was preferred to biacetyl; a laboratory-prepared butter flavor containing monobutyrin, butyric acid, and biacetyl gave similar results.

Factors Influencing Biacetyl Content of Butter. The biacetyl content of butter is influenced by various factors; these begin with quality of the cream and continue through washing of the butter. Commonly, a high or low biacetyl content accompanies a high or low acetylmethylcarbinol content.

Quality of Cream. Sweet cream may contain biacetyl and acetylmethylcarbinol, the amounts presumably being influenced by the feed. During the souring of cream these compounds commonly are produced, often in relatively large amounts, but objectionable changes also may occur and make the cream less satisfactory for butter than it was before the souring.

Neutralization and Pasteurization of Cream. According to Barnicoat,[45] neutralization and pasteurization of cream caused no notable decrease in biacetyl but a distinct decrease in acetylmethyl-

carbinol. Destruction of the carbinol was correlated with the neutralization point. Davies [46] noted that cream which had developed biacetyl and acetylmethylcarbinol did not yield butter having flavor and aroma when the cream was neutralized and flash pasteurized. Disappearance of the compounds from the cream was explained on the basis of their volatility and absence of oxidation of the carbinol in the butter. Prill and Hammer [47] found marked changes in biacetyl contents, both decreases and increases, during neutralization and pasteurization of sour cream; changes in the carbinol were relatively small. It appears that generalizations on the effect of neutralization and pasteurization of cream on biacetyl and carbinol contents cannot be made because of variations in the original cream and the treatments given.

Use of Culture. Proper use of good culture definitely increases the biacetyl and acetylmethylcarbinol contents of butter. Action of culture in tending to control certain spoilage organisms that occasionally gain entrance to butter also is an advantage in its use.

Addition of Citric Acid. The effect of added citric acid in increasing the biacetyl and carbinol contents of lactic cultures indicates that under certain conditions its addition to cream intended for butter would be useful. Evenhuis [48] concluded that it is highly desirable to have some citric acid in fresh butter. Adding citric acid to the cream appears to be of special value in the manufacture of unsalted butter from sour cream since the bacteria in such cream have lowered the citric acid content from that originally present.

Aeration. The influence of oxygen in the fermentation of citric acid by culture organisms indicates that a good oxygen supply would favor biacetyl production in cream.

Agitation during Churning. Agitation of cream and butter granules in the presence of air during churning would be expected to have an effect on the biacetyl and carbinol contents. Mazé [49] noted that churning must be regarded as an additional aerobic fermentation which tends to oxidize the products of the anaerobic fermentation and produce the flavor characteristic of first-quality butter. Virtanen [50] stated that churning of cream in a churn filled with inert gas obviously is not suited to production of butter with a rich aroma; he suggested churning in such a manner as to ensure a maximum content of air in the butter since this promotes formation of carbinol and biacetyl. Analyses also indicate an increase in biacteyl during churning.[47]

Partition of Biacetyl and Carbinol during Churning. Butter contains much less biacetyl and carbinol than the cream from which

it was churned, and the buttermilk contains relatively large amounts. Percentage retentions in the butter reported by different investigators vary rather widely, and variations also occur in tests by individual investigators, perhaps due to differences in conditions, especially the amount of serum held in the butter.

Barnicoat [45] reported that 0 to 33% (average 15) of the biacetyl and 7 to 22% (average 14) of the carbinol in the cream were retained in the butter. Prill and Hammer [47] found that on the average 8.8% of the biacetyl and 4.5% of the carbinol plus biacetyl in the churning mixture were retained in the butter.

Washing of Butter. Various investigators have noted that, during washing, butter loses flavor and that this is reflected in the biacetyl contents of the butter and wash water. Prill and Hammer [47] found that butter wash water contained only a fraction of a milligram of biacetyl and carbinol per kilogram. They considered that, although the total content of these compounds in wash water may be significant, compared to that present in butter, it is difficult to differentiate between material that comes from the film of buttermilk remaining between the granules and that which might be removed from the granules themselves.

Partition of Biacetyl and Carbinol between Fat and Serum of Butter. Because of the comparative amounts of biacetyl and carbinol in butter and buttermilk, the serum of butter would be expected to contain larger amounts of the compounds than the fat. Various investigations confirm this relationship. Hoecker and Hammer [51] reported that the serum of butter contains higher concentrations of the compounds than the fat, with the greater difference involving the carbinol. Percentages of biacetyl and carbinol in the fat were smaller with unsalted butter than with salted. With biacetyl the percentage in the fat was independent of the concentration in the butter, whereas with the carbinol it tended to decrease as the concentration in the butter increased.

Changes in Biacetyl and Carbinol Contents during Holding of Butter. During the holding of butter there are various possibilities in connection with changes in biacetyl and carbinol contents, such as increases through action of culture organisms and decreases through action of bacteria or chemical reactions. Various factors influence these possibilities and may explain the variations from one lot of butter to another. Since culture organisms are sensitive to salt, with appreciable salt concentrations in butter they probably are not active. However, there may be points at which there is little or no salt, and here

the organisms may be active; this could account for the increases in biacetyl in salted butter that have been reported. Commonly, the *p*H of salted butter is too high for significant biacetyl production. In such butter biacetyl and carbinol seem to be rather well retained and a less satisfactory flavor on holding may be due to development of undesirable flavors rather than to loss of biacetyl.

In the case of unsalted butter made with ripening of the cream, significant changes in biacetyl and carbinol occur at temperatures permitting growth of the culture organisms. Both increases and decreases have been noted. Various factors undoubtedly are involved, such as *p*H, presence of citric acid, activity of culture organisms, etc. It appears that an increase in biacetyl content of unsalted culture butter is important in the flavor development on holding which this type of butter often shows.

Prill and Hammer [52] noted that, in general, salted (about 2.25%) butter made with different manufacturing procedures showed only slight changes in biacetyl and carbinol contents when held frozen or at 36 to 45 F. Even at 70 F, at which temperature chemical changes in butter are especially rapid, the compounds were largely retained for considerable periods. With unsalted butter in a frozen condition, changes in the contents of the compounds were relatively slight but at 36 to 45 F or 70 F significant changes occurred, both increases and decreases being noted.

Undesirable Flavors in Butter

Undesirable flavors in butter can be divided into two groups, (1) those present in fresh butter, and (2) those that develop on holding. Various factors are responsible for these flavors, but the products formed by organisms, either in the cream or the butter, are directly or indirectly concerned with a number of them.

Causes of Undesirable Flavors in Fresh Butter. The flavors in fresh butter are closely related to the flavors in the cream as it goes to the churn. Certain flavors are more pronounced in the butter than in the cream through their association with the fat; others are less pronounced because they are associated chiefly with the nonfatty constituents and are largely carried away in the buttermilk. Washing butter tends to decrease the intensity of some flavors. Salt brings out many flavors in butter so that certain undesirable flavors definitely are more conspicuous in salted butter than in unsalted butter from the same lot of cream.

The flavors in cream as it goes to the churn are determined by the

flavors in the cream coming to the plant and by the methods used in the plant. The principal causes of undesirable flavors in sweet cream are the feed and condition of the animals; with sour cream action of organisms is the most important. The principal defects in the plant methods that influence the flavor of butter are improper neutralization or pasteurization and culture of poor quality.

Causes of Undesirable Flavors that Develop in Butter. Although the flavor of butter may improve for a short period after its manufacture through a decrease in some such condition as a conspicuous heated flavor, or through intensifying of the desirable flavor, the general effect of holding is a decrease in quality. Extent of the decrease varies greatly; in butter properly made from high-quality cream and stored at suitable temperatures it may be negligible, but in a poorly made product stored under unfavorable conditions it may be very serious. Changes in butter that result in development of undesirable flavors involve bacterial, enzymatic, and chemical action. In considering the flavors that develop in butter during holding, ability of the product to absorb odors must be recognized.

FACTORS INVOLVED IN BUTTER DETERIORATION

Differentiation of bacterial, enzymatic, and chemical changes in butter deterioration sometimes is difficult. Temperatures and general conditions that favor development of bacteria also may accelerate action of enzymes, and some of them may favor certain chemical changes. In attempting to correct a specific flavor defect in butter, the first step is to determine its cause, that is, whether it is bacterial, enzymatic, or chemical. Often this is evident from the nature of the defect and corrective measures are formulated easily, but in other cases there is more than one possibility and the problem requires investigation. Study of butter deterioration is complicated by the necessity of basing conclusions as to the extent of the change on the senses of smell and taste, rather than on definite measurements.

Bacterial Action. Early studies on the bacteriology of butter were suggested by the frequency with which the product deteriorated and by the importance of organisms in causing changes in milk. Unsalted butter made from *raw* cream normally undergoes bacterial deterioration when held at temperatures permitting growth of organisms; various defects develop, especially cheesiness and rancidity. Appearance of the defects can be delayed, or prevented, by limiting growth of the causative organisms through low temperatures, salt, etc. Proper pas-

teurization of cream is recognized as an important factor in manufacturing butter that will keep satisfactorily when held at temperatures prevailing during its distribution and consumption.

Many species of bacteria are capable of bringing about spoilage in butter. For the most part they are capable of growth at relatively low temperatures; also, they are types that attack protein and/or fat and often have little effect on lactose. In general, they are not resistant to heat and when butter made from pasteurized cream, with or without salt, shows bacterial deterioration, the causative organisms normally are present because of inefficient pasteurization and/or contamination subsequent to heating.

At one time there was a definite tendency to ascribe all butter deterioration to action of bacteria. Evidence gradually accumulated which suggested that butter often deteriorates under such conditions that bacterial action is improbable. Experimental results also showed that butter could deteriorate at fairly high temperatures without an active increase in organisms.[53]

Data from various sources indicate that, when butter is held at *low* temperatures, addition of organisms to the cream from which it is made has little influence on the keeping quality. Rogers *et al.*[54] compared butter from (1) raw cream, (2) pasteurized cream, and (3) pasteurized cream inoculated and held so that after 24 hr the numbers and types of the organisms approximated those before pasteurization without an appreciable increase in acidity having occurred. The reinoculation of the cream had little or no effect on the keeping quality.

Products of bacterial growth, especially acids, may greatly influence certain chemical changes in butter so that organisms play a role in butter deterioration both directly and indirectly.

Enzymatic Action. Cream to be made into butter contains the enzymes normally present in milk and also the extracellular enzymes that may have been produced by organisms growing in it, or in the milk from which it came. Some of these enzymes bring about the types of changes that are important in butter deterioration, and they may be of importance under certain conditions. Pasteurization destroys some of the enzymes and decreases the activity of others.

Rancidity is a common defect of raw-cream butter, such as still is made in small amounts on farms, and in many instances activity of milk lipase appears to be involved. Because of the ease of its destruction by heat, it is of no importance in butter made from properly pasteurized cream.

Rogers *et al.*[54] found that in raw-cream buttermilk, to which chloro-

form had been added, activity of protease was almost entirely inhibited by the presence of 18% sodium chloride and a temperature of 0 F; when some of the same material was held at room temperature, the protease apparently became more active because the casein clotted and the mixture assumed an appearance suggesting digestion. A trial with a bacterial enzyme preparation obtained from a proteolytic, acid-forming organism indicated that milk proteins were hydrolyzed at 20 F in the presence of a high sodium chloride concentration. Combs and Eckles [55] concluded that the abnormal flavors which developed in butter as a result of mold growth on the cream were caused by enzymes secreted by the molds. *G. candidum* and a *Penicillium* were used alone and in combination. Pasteurization at 145 F for 20 min, or 165 F for 5 min, greatly improved the keeping quality of butter made from cream on which molds had grown but did not entirely check the action of the enzymes which had been produced.

Thatcher and Dahlberg [56] reported that the normal milk enzymes need not be considered a factor in the deterioration of butter during storage because of the small amounts in which they are present and the unfavorable influence of storage temperatures on enzyme activity; the investigators found that sodium chloride inhibits protease to such an extent that the enzyme cannot act in normal salted butter.

Chemical Action. Inadequacy of the action of organisms as an explanation of butter deterioration under all conditions gradually led to the conclusion that chemical changes are important. Under storage conditions they may be the principal cause of deterioration, and at higher temperatures they may be relatively less important because of the influence of other factors, although actually more extensive.

Influence of Air. Relationship of air to butter deterioration long has been suspected because of the frequency with which the surface portion of print or bulk butter shows a less desirable flavor than the interior. Air tends to favor growth of certain organisms in butter and may influence deterioration in this way, but such an effect would not be operative at low temperatures and chemical action is indicated. Gray [57] found that under various conditions of storage, including storage at −10 F, butter in full cans kept much better than in cans partly full and ascribed this to the presence of air in the unfilled cans. Dyer [58] studied oxidation in butter by analyzing the gas present after varying periods of holding; trials were carried out with butter made in various ways and kept at different temperatures. He concluded that the production of off flavors so commonly met with in cold-storage butter is attributable to a chemical change expressed through slow

oxidation that is progressive in one or more of the nonfatty constituents. Extent of the change was proportional to the quantity of acid present in the cream from which the butter was made. With fat alone, oxidation was slight; with buttermilk it was rapid when acid was present, but slight in the sweet product.

With a churn adapted to working butter under reduced air pressure, Barnicoat [59] prepared lots of butter with reduced "free air" contents. The free air content of normal samples averaged 5.8 ml per 100 g whereas samples worked under 15 in. vacuum averaged 3.6 ml after 2 min and 1.2 ml after 15 min. Working 15 min under 20 in. vacuum removed all free air from sweet-cream butter and 85% from ripened-cream butter. Reduction of free air content reduced the butter bulk by 5 to 6% and produced a smooth, close texture. There was no improvement in flavor or keeping quality of either sweet- or ripened-cream butter, or in retarded fat oxidation.

Influence of Acid. At one time it commonly was believed that development of acid in cream intended for churning gave the butter more satisfactory keeping quality. Presumably, this idea resulted from the ease with which acid foods can be preserved. Following earlier suggestions,[60,61] Patrick *et al.*[62,63] carried out trials and found that sweet-cream butter deteriorated less than that made from ripened cream. These early experiments are of special interest because of the comparatively high storage temperature, approximately 50 F, used in most of them. In a study of fishy butter, Rogers [64] found that in all cases where the records were complete the experimental butter that became fishy was made from high-acid cream. Rogers and Gray [65] concluded that butter frequently undergoes marked changes, even when stored at very low temperatures, and that these changes are more marked with an increase in acidity of the cream from which the butter is made; changes in the high-acid butter were not checked by heating the ripened cream. Additional early trials [66,67] emphasize the role of acid in the chemical deterioration of butter.

White [68] reported results showing the deterioration of butter made from sweet cream and from cream with various acidities, the lower acidities being obtained by addition of culture, whereas for the higher acidities the cream was ripened. All the cream was pasteurized at 145 F for 30 min; in some cases pasteurization was carried out after adding the culture, but this did not appear to have any material influence on the results. There was very little deterioration in the butter made from cream having 0.15 or 0.25% acid; when the cream had

0.31% acid, deterioration was slightly greater and, when it had 0.35% acid, or more, deterioration was pronounced.

Studies involving *p*H determinations on butter serum also indicate the influence of acid on chemical changes in butter, low *p*H values occurring with butter that keeps poorly in storage.[32,69,70] White[71] noted that acidity of the cream and therefore *p*H of the butter serum had a definite effect on the flavor scores of butter. As the average acidity of the cream increased from 0.11 to 0.20 or 0.31%, the average *p*H of the butter serum decreased from 6.76 to 6.1 or 5.51, respectively, with a resulting loss in flavor scores of 2 to 3 points. In medium- and high-acid butter, the principal flavor defects were metallic, sour, or acid, and in one case fishiness.

Other experimental data and extensive commercial experience emphasize the importance of low *p*H values in the deterioration of salted butter on holding. Although the *p*H ranges suggested as the most desirable differ somewhat, the danger in low values is generally recognized. The desired *p*H in butter serum is obtained by neutralization of the cream and, with proper procedures, the *p*H can be controlled within narrow limits. Various butter organizations regularly check the *p*H values of butter to be certain that the neutralization is being carried out properly.

Acid influences the deterioration of butter primarily through its effect in increasing the rate of chemical changes. Since certain lots of butter with low *p*H values keep satisfactorily, it is probable that contributing factors are involved; these may vary from one lot of butter to another.

Influence of Metal. Results of early investigations indicated that added metals favor butter deterioration. Rogers *et al.*[54] found that addition of iron to cream, even in such small amounts as 1 or 2 ppm, had an influence on the butter; the nature of the off flavor developed on holding was not changed appreciably but rate of development was accelerated. Action of copper was similar but perhaps more intense. In general, only a relatively small part of the iron goes into butter as compared with buttermilk and, accordingly, a flavor due to iron was most noticeable in the buttermilk. A large percentage of the iron retained by the butter was present in the curd. The investigators also found that a strong odor could be produced in milk by addition of small amounts of iron salts, the ferrous salts being more active than the ferric. More recent investigations also emphasize the influence of metals in bringing about deterioration in butter, and copper rather regularly has been more active than iron. Loftus-Hills *et al.*[33] re-

ported that butter acidity and copper content appeared to be the most important factors controlling keeping quality in 70 boxes of salted butter from Australia.

The conspicuous defect caused by an excessive amount of copper in butter commonly is tallowiness, which may be followed by bleaching; these defects eventually appear in all butter so that actually the copper simply speeds their development. In certain instances copper has caused fishiness. Apparently, when the effect of the copper is less pronounced, the off flavor produced cannot be definitely characterized.

During the handling of milk and cream, small amounts of iron or copper may be added to them. Tinned iron or copper equipment often has the structural metal exposed, as the result of wear or accident, and then iron or copper may be picked up by milk or cream handled in it, through mechanical removal or formation of salts. The amount of metal picked up is influenced by various factors, such as condition of the equipment, washing procedures used with it, reaction and conditions of exposure of the product, and exposure to air. Such metal contamination is being controlled rapidly through use of stainless steel and other materials for dairy equipment.

The iron and copper occurring naturally in milk and cream vary in amount, due to feed and other factors, but they apparently are much less important as a cause of butter deterioration than the iron and copper added during the handling.

Influence of General Composition. Although it appears that the variations encountered in moisture and curd contents of butter under commercial conditions have no significant influence on the rate of deterioration, the presence of these constituents in butter results in a decrease in keeping quality, compared to more highly purified fat. The ghee made in various countries by heating unsalted butter and then straining it is a product that keeps well under severe temperature conditions; the heating largely evaporates the water, and the straining removes a part of the curd which the heat tended to flocculate. Patil and Hammer [72] found that both ghee and butterfat kept much better than ghee with water added or than butter. Addition of water to ghee accelerated deterioration more than addition of protein.

Nonwashing of Butter. Washing of butter is standard practice in most butter plants. Recently, certain plants have made butter without washing, in some cases because of uncertain water supplies, and the procedure is receiving increased attention.

It long has been known [73] that washing removes part of the lactose and curd from butter. Wyant [74] reported average curd contents for washed and unwashed butter of 0.38 and 0.52%, respectively; Pont [75] found values of 1.10 and 1.52% and later [76] of 0.99 and 1.43%; and Arnott *et al.*[77] obtained values of 0.8 and 1.5%.

The differences in quality and keeping quality between washed and unwashed butter are not sharply defined but there appears to be a tendency in favor of the washed product. Perhaps cream quality and other factors influence this. Diatchenko [78] concluded from analyses of European butter that curd content is not an important factor in keeping quality of the product. Arnott *et al.*[77] found that *p*H values of washed and unwashed butter were in the same range at time of manufacture and did not change appreciably during storage; also, there was no essential difference from a bacteriological standpoint between washed and unwashed butter after storage. Pont [76] noted that bacterial and yeast counts are higher on unwashed butter. It appears that under many conditions washing of butter can be omitted and that it is preferable to the use of a questionable water supply.

Effect of Carbonation. In some instances, butter has been churned and worked in an atmosphere high in carbon dioxide. Results of various investigators indicate that the procedure has no significant effect on bacteria and does not improve the flavor or keeping quality of butter.[79, 80, 81]

SPECIFIC FLAVOR DEFECTS OF BUTTER

Specific flavor defects of butter can be divided into three groups: (1) Those due primarily to off flavors in the cream, (2) those due primarily to unsatisfactory plant methods, and (3) those that develop primarily during the holding of butter. The division is not clear cut and a flavor may appear under conditions that class it with a group other than the one to which it ordinarily belongs; for example, a fishy flavor most often develops when butter is held but, occasionally, is present in fresh butter. The grouping is useful, however, in that it emphasizes the important causes of off flavors in butter.

Defects Due Primarily to Off Flavors in the Cream

Various off flavors in the cream, whether due to organisms or other causes, may be carried into the butter. Some of these are removed in part, or rather completely, by pasteurization, particularly vacuum pasteurization, and/or neutralization; others definitely are not.

Feed Flavors. Various feed flavors in butter are related directly to similar flavors in the cream. The most serious of these are due to onions, garlic, french weed, peppergrass, etc., but certain very useful feeds, such as silage and legumes, may affect the flavor of butter.

Barny Flavor. A barny flavor in cream, due to absorption of odors in the stable, often is carried into butter. A somewhat similar condition in cream occasionally is due to the growth of organisms, especially certain of the *Aerobacter* species.

Malty Flavor. A malty flavor produced in cream through growth of organisms is readily transferred to the butter. Since cream may show a malty flavor before there has been a significant increase in acid, the condition is noted at plants receiving either sweet or sour cream.

Old Cream Flavor. Certain lots of butter show a flavor that is difficult to describe but suggests that the cream used was of poor quality because of growth of organisms. It frequently is designated old cream flavor and definitely is due to growth of organisms in the cream.

Yeasty Flavor. A yeasty flavor in butter is due primarily to the development of the lactose-fermenting yeasts, *C. pseudotropicalis* and *T. sphaerica,* in the cream before it reaches the plant, although careless holding of the cream in the plant sometimes results in a yeasty flavor or intensifies it if already present. The typical yeasty flavor is noted only in sour cream butter.

Musty Flavor. Cream may have a musty flavor as a result of storage in a cellar or cooling tank having a musty odor, or molds may grow on cream subjected to favorable growth temperatures for extended periods; under these conditions the butter often has a musty flavor.

Rancid Flavor. A rancid flavor, due to activity of lipolytic organisms or milk lipase, sometimes is present in cream and is readily carried into butter. The flavor can be reduced by proper neutralization.

Cheesy Flavor. One of the most objectionable flavors in butter due to the condition of the cream is a cheesy flavor. Presumably, various organisms can produce cheesiness in cream if they grow in it for considerable periods, but extensive growth of lactobacilli is especially important in this connection. A cheesy flavor due to the condition of the cream definitely should be distinguished from cheesy flavors that result from the development of organisms in butter.

Metallic Flavor. A flavor that is referred to as metallic sometimes is present in butter. It also is common in buttermilk. The flavor suggests the astringent effect given by certain metallic salts when taken into the mouth. In butter, it is due primarily to the same defect

in the cream at churning. The flavor may be present in cream as it comes from the producing farms or may develop during holding in the plant. A metallic flavor in cream often disappears when the cream is held under conditions favorable for growth of organisms. Because of its relationship to the condition of the cream, a metallic flavor in butter usually is present as soon as the butter is made.

Absorption of metals appears to be one cause of a metallic flavor in cream. The defect is intensified by acid, and cream next to some metal surfaces, especially exposed copper or iron, may have a pronounced metallic flavor. However, Mulder *et al.*[82] considered that the metallic and metallic-oily flavors in cream and butter are not caused by metallic salts but are due to oxidation of fat or fat-like substances in the cream.

Metallic flavor also appears to be due to the action of certain bacteria. Guthrie[83] studied the development of metallic flavor in dairy products and concluded that direct absorption of metals or action of bacteria may be responsible. Under suitable conditions, especially when the acidity is high, the normal lactic culture organisms apparently can produce metallic flavor in various dairy products.[84]

Metallic flavor in butter usually is soon replaced by some other flavor and is uncommon in storage butter. Fishiness often follows it and, since excessive acid in the cream is related to fishiness, the importance of acid in causing metallic flavor is suggested. Proper neutralization of the cream is an important factor in controlling metallic flavor in butter.

Flavors Distinctly Foreign to Butter. Butter occasionally shows a flavor distinctly foreign to it, such as kerosene, gasoline, fly spray, or disinfectant. This usually is due to absorption of odors by the cream, but there may be an actual addition of some foreign material through carelessness.

Defects Due Primarily to Unsatisfactory Plant Methods

Cooked Flavor. Fresh butter made from pasteurized cream commonly has a slight cooked flavor, but this largely disappears within a few days. In some instances, however, the cooked flavor is pronounced and persists during the holding so that it definitely is objectionable.

An excessive pasteurization exposure for all or a part of the cream is the primary cause of the cooked flavor. With an excessive temperature at the heating surface, there is a tendency for the cream in direct contact with it to be overheated; when cream adheres to the heating

surface and is subjected to high temperatures for extended periods, the cooked flavor is especially pronounced.

Neutralizer Flavor. Butter made from neutralized cream often shows more or less of a neutralizer flavor. Its extent is related to the amount of neutralizer required to reduce the acidity to the desired point and also to the method of use. If the neutralizer is not properly distributed, if the neutralization is carried too far and the cream then pasteurized, or if the cream is heated to a comparatively high temperature before the neutralizer has reacted with the acid present, an undesirable flavor effect is to be expected. There is no close relationship between the *p*H of the serum and a neutralizer flavor in butter. Brown and Parfitt [85] reported that the *p*H values of 892 samples of butter criticized as having a slight or distinct neutralizer flavor ranged from 4.15 to 7.96.

Flat Flavor. Butter made with culture so that it should show a full flavor sometimes does not and is referred to as flat. In general, this is the result of culture of poor quality, although in some cases a desirable flavor disappears from butter as a result of destruction of the biacetyl originally present by organisms or chemical reactions.

High-acid or Sour Flavor. A flavor suggestive of considerable acid sometimes is noted in butter. Commonly, it is the result of excessive acidity in the cream at churning but also may be due to the condition of the culture. When a culture simply forms acid, instead of giving a typical flavor, there is a tendency for butter in which it is used to show the defect.

Defects that Develop Primarily during the Holding of Butter

Storage Flavor. Butter that has been in storage for a considerable period commonly shows a flavor that the experienced butter grader recognizes as storage flavor. The intensity varies greatly and, with properly made butter, is slight. The condition probably is due to slow chemical changes; although such changes are reduced to a minimum by high-quality raw material and proper manufacturing methods, they cannot be prevented entirely.

Rancidity. A rancid flavor sometimes develops in butter. The condition shows great variation and may be so modified by other off flavors that its detection is difficult. In some cases the products of hydrolysis are later changed, presumably through action of organisms or chemical reactions.

Micro-organisms constitute an important cause of fat hydrolysis in butter which results in rancidity. In the early studies of Orla-

Jensen [86] a number of species were found which produced rancidity, including *G. candidum, Cladosporium butyri, Ps. fluorescens,* and *Ser. marcescens.* Other organisms also can produce the change. *Ps. fragi* has been detected repeatedly in rancid butter in numbers sufficiently large to indicate its causal relationship to the defect.

If it has an opportunity to act on butter, milk lipase may cause rancidity. In some instances butter made in commercial plants from raw cream, because of failure of the pasteurization equipment, has developed rancidity without showing significant numbers of lipolytic organisms.

Jack *et al.*[87] separated cream from milk that developed rancidity on holding, and also from normal milk, and churned it. Provided the milk was pasteurized within 18 hr after it was drawn, the butter made from either type of cream and stored at 20 to 22 F did not deteriorate up to 60 to 65 days, as shown by flavor score of the butter and acidity of the fat. When either type of cream was held raw at 40 F for 3 days before pasteurization, lipolysis occurred to the detriment of the resulting butter; the butter from milk that developed rancidity on holding was inferior to that from normal milk.

Organisms and lipase are the primary causes of rancidity in butter. Guthrie [88] noted that exposure of butter and butterfat to high temperatures, light, and air did not cause rancidity. In general, the organisms that cause rancidity in butter are easily destroyed by heat and, since lipase also is readily inactivated, proper pasteurization and protection against recontamination make it possible to produce butter that will not become rancid.

Fishiness. Development of a fishy flavor in butter is one of the very serious types of deterioration. In the United States it is primarily a defect of salted butter and is rare in the unsalted product. Fishiness occurs mainly in butter that has been stored, but occasionally appears in fresh butter when the acidity at churning is relatively high. Some of the heavy financial losses in storage butter have been due to development of pronounced fishiness in butter that reached storage with excellent flavor.

The early investigations on fishiness led to various conclusions. O'Callaghan [89,90] reported results which suggested that *G. candidum* was the cause of the defect, but other studies failed to confirm this. The idea that salt may cause fishiness was considered by Piffard [91] who pointed out the ability of salt to absorb the odor of material stored near it.

Rogers [64] concluded from his study of fishy butter that the flavor

evidently is not produced by the action of any one special factor and that its immediate cause is a substance produced through oxidation of one of the combinations of the acid developed in the ripening of cream. He noted that acid is essential and that butter made from pasteurized sweet cream with culture, but without ripening, seldom if ever becomes fishy; overworking butter made from sour cream produced a fishy flavor with reasonable certainty. Reakes *et al.*[92] were unable to find significant differences in the bacteria of fishy and high-grade butter and concurred in the idea that a chemical change results in a splitting of some butter constituent into compounds having a fishy flavor.

The fishy odor of trimethylamine naturally suggested this compound as a cause of fishiness in butter. Although Rogers[61] was unable to produce fishiness by working large quantities of it into butter, Supplee[93] obtained a fishy flavor when trimethylamine was worked in under proper conditions, especially when trimethylamine butyrate was used, and noted that normal butter contains lecithin, which can yield trimethylamine on decomposition. Sommer and Smit[94] reported that addition of trimethylamine lactate to butter caused a distinct fishy flavor and that trimethylamine could be isolated from samples of fishy butter. When made from cream to which lecithin had been added, butter became fishy more readily than when made from untreated cream of the same lot. The investigators noted that organisms which produced trimethylamine from lecithin under favorable conditions failed entirely to grow in the presence of salt and acid in the concentrations found in the moisture of butter.

With chemical decomposition of lecithin the cause of fishiness, the factors influencing this process become of importance. Acid favors the hydrolysis of lecithin and may increase the content of metals that can act as catalysts. Salt solution is a solvent for lecithin and thus may change it to such a condition that chemical decomposition can go on more rapidly. Overworking favors the solution of the lecithin and may distribute the incorporated air more uniformly. Pasteurization tends to eliminate fishiness[94] because it causes some hydrolysis of the lecithin, the products of which are lost in the buttermilk, and because during heating the lecithin absorbs oxygen and becomes less soluble.

Cusick[95] reported that fishy flavor was produced in butter by inoculating the cream with *Ps. ichthyosmia.* Age and acid content of the cream and period of incubation before churning appeared to influence production of the flavor in the butter. In a trial carried out

by Horrall [96] lecithin disappeared much more rapidly in salted butter made from high-acid cream than in salted butter made from sweet cream. However, none of the butter was criticized as fishy, which suggests that lecithin decomposition does not always yield trimethylamine.

Hussong *et al.*[97] found that fishy butter from various plants commonly had lower *p*H values than nonfishy butter from the same plants at about the same periods. Butter from one plant developed fishiness at higher *p*H values than that from the other plants, suggesting that a factor other than *p*H also was operative. In various instances in which fishy butter had a relatively high *p*H, it contained comparatively large amounts of copper. It was noted that cream from certain producing areas apparently yields butter that is more susceptible to development of fishy flavor than cream from other areas.

Although fishiness is much less common than formerly because of the relatively low churning acidities now used, it still is a serious defect. With storage butter the intensity often greatly increases during the period the butter is in retail channels and it appears that when the chemical change resulting in fishiness is once begun it continues at an accelerating rate. In the control of fishiness the first consideration should be the *p*H of the butter serum. Hussong *et al.*[97] noted that when cream comes from a variety of sources control of the *p*H may be difficult because of some unusual relationship between titration of the cream and *p*H of the butter serum. They studied a plant in which the butter began to develop a fishy flavor following receipt of considerable volumes of cheese-factory cream, both whey and whole-milk cream. The *p*H values of the butter were relatively low although the churning acidities had not been changed. Eventually, it was learned that certain cheese factories were skimming a rich cream and then diluting with cold water to cool the cream and thin it sufficiently to make handling easier. Addition of the water changed the buffer capacity of the cream so that the *p*H at a given acidity was considerably lower than it otherwise would have been. If the *p*H of fishy butter is 6.8, or above, the possibility of excessive copper contamination should be considered. With cream from a variety of sources this also involves the possibility of some unusual situation, such as excessive copper contamination from equipment.

Putrid or Proteolytic Butter, Cheesiness, Surface Taint, and Rabbito. Butter shows a series of closely related off flavors and odors that range from a slight abnormality through defects which suggest various cheeses, tainted protein, or a putrid condition. The off flavors com-

monly develop quickly. Often they appear first at the surface of butter and are most conspicuous there, but eventually they are evident throughout the mass. Various explanations have been suggested for the defects but action of bacteria in the butter is responsible; the general nature of the bacteria suggests that the primary effect is on the protein. *Ps. putrefaciens* appears to be the common causative species. When it develops in butter, the defect first is slight, then

Figure 38. Bacteria in putrid butter. Courtesy R. V. Hussong.

becomes more and more objectionable, and finally is less conspicuous.

Eckles [98] reported an outbreak of putrid butter that occurred in 1900 and lasted about 2 weeks. The defect was so pronounced the local sale of butter was stopped. The plant was in good condition, but the milk received was of unsatisfactory quality. Changes involving rejection of poor-quality milk, better care of utensils, and use of lactic culture were instituted at the plant, and about the same time a long, dry period was broken by heavy rains. The butter improved at once and no further difficulty was experienced.

In Canada, a butter defect commonly described as surface taint has been encountered frequently. According to Hood and White,[99] it made its appearance, in 1919, in first-grade pasteurized-cream butter sent to the Vancouver market. The investigators noted that butter with this defect showed abnormally high yeast and bacterial counts

and that all the samples contained large numbers of bacteria capable of decomposing curd. Surface taint was produced in experimental butter from pasteurized, neutralized cream by inoculating into the cream certain bacteria isolated from water.

Sutton [100] reported that two striking characteristics of a decomposed odor in butter are the rapidity with which it may develop in butter apparently of satisfactory quality and its lack of correlation with the numbers and types of bacteria which develop on beef extract agar.

Derby and Hammer [101] found that surface-taint butter often contained large numbers of bacteria. Counts were higher at the surface of a sample than in the interior in all but 1 of 20 comparisons. Surface taint could be produced by inoculating pasteurized cream with the defective butter and churning. Two to 4 days were required for the defect to develop at 60 F and 7 to 10 days at 41 F. The defect sometimes developed in commercial butter held at temperatures favorable for bacterial growth. The organisms that predominated on beef infusion agar plates poured with surface-taint butter did not produce surface taint on inoculating into pasteurized cream and churning. An organism which regularly produced the defect in unsalted butter first was isolated from Canadian butter; it was designated *Achromobacter putrefaciens* but now is included in the genus *Pseudomonas.* Later, it was obtained from additional samples of surface-taint butter. The organism was greatly restrained by use of medium percentages of salt or by culture.

Experience in various laboratories indicates that special isolation procedures are needed in examining butter for *Ps. putrefaciens.* Claydon and Hammer [102] isolated it from commercial putrid butter most easily by inoculating the butter into thoroughly pasteurized cream, churning, and smearing the butter on agar after the defect had developed. Inoculation of the defective experimental butter into cream for production of second or third generation samples occasionally resulted in isolation of *Ps. putrefaciens* when it had not been obtained previously. Incubation of the plates at 41 to 50 F (5 to 10 C) also aided in the isolation.

For the isolation of *Ps. putrefaciens,* Linneboe [103] used nutrient agar at *p*H 7.2 to 7.5 and, before pouring the plates, added sterile ferric ammonium citrate at the rate of 0.5 ml of a 10% solution per 10 ml of agar. The plates were incubated 7 days at 77 F (25 C). Any well-isolated colony that had made a depression in the medium varying in extent from slightly sunken to one resembling gelatin liquefaction was studied for final identification.

Long and Hammer [104] developed a medium for isolation of *Ps. putrefaciens* that consisted of 4% gelatin, 1.5% agar, 2% proteose peptone, 0.1% dipotassium phosphate, 0.05% ferric ammonium citrate, and water to make 100%. Smearing the plates was better than pouring in the usual way. The organism often develops readily on the medium, and

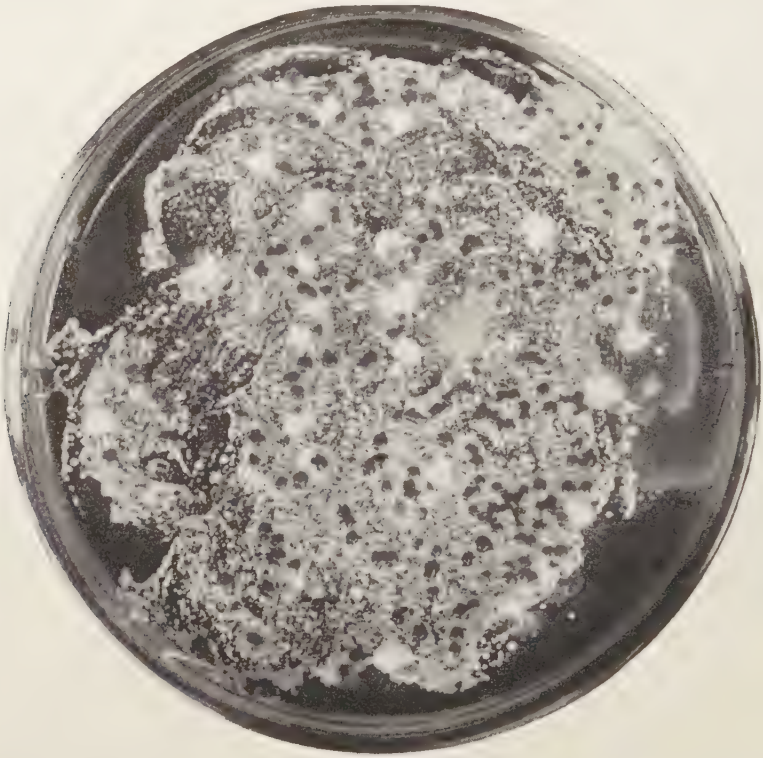


Figure 39. Large colonies of *Pseudomonas putrefaciens* among smaller colonies of other species on special medium of Long and Hammer.

after several days colonies are fairly large, raised, and brown to reddish brown or pink; however, growth may not occur when the number of organisms in the inoculating material is small and certain strains appear to have difficulty in developing. In isolations from butter, the serum was more satisfactory than the butter direct. With various materials, enrichment in litmus milk at 37.4 F (3 C) frequently was helpful.

Working with pure cultures of *Ps. putrefaciens*, Reinbold *et al.*[105] found that certain amino acids, aspargin, numerous complex hydro-

lyzed proteins, and intact protein materials were utilized. Hydrolyzed proteins afforded the best growth of the test cultures.

Ps. putrefaciens is widely distributed and has been isolated from water supplies, floors, sewers, and equipment, including churns, insulation of leaky vats, etc. At certain points in equipment the organisms may be present in considerable numbers. Cullity and Griffin [106] investigated outbreaks of rabbito in Australian butter and emphasized the importance of water as a source of the causative organism. Pont [107] noted that the original source of infection of butter with rabbito organisms in the plant appears to be the water supply but that secondary, and in some cases more serious, foci of infection may be set up in churns and other equipment, especially when made of wood. Heavy salting, high acidities, thorough working, and efficient manufacture assist in minimizing or controlling the defect, but these factors cannot be relied upon for complete control in the presence of adequate infection and suitable growth temperatures. Itzerott [108] reported that rabbito occurred most frequently in butter showing open texture and free moisture. He also noted that butter with a heavy infection of rabbito organisms showed no sign of the defect when stored for 3 months, but on removal from storage developed the defect, usually in 30 to 40 hr at 65 to 95 F. Previously, it had been shown [34,106] that reworking of apparently normal butter may rapidly result in the putrid defect.

Thomson [109] examined 1,563 samples of normal butter (generally 1.5 to 2% salt) and isolated *Ps. putrefaciens* from 91. There appeared to be a close relationship between poor factory sanitation and the presence of *Ps. putrefaciens*. The salt content and moisture distribution may prevent its development during storage, whereas the high temperatures in retail shops encourage its growth.

Long and Hammer [104] sealed various strains of *Ps. putrefaciens* grown in milk for 1 and 7 days in glass tubes and exposed them in a water bath at 143 F; all cultures survived for 0.5 min but most of them were killed in 1 min and the remainder in 2 min. In water, *Ps. putrefaciens* appears to be destroyed readily by chlorine when the number of organisms is not excessive in relation to the chlorine concentration or its period of action.

Elliker and Horrall [110] found that the first apparent stage of decomposition when *Ps. putrefaciens* developed in either commercial or laboratory butter was complete loss of typical butter aroma; the butter became flat and, later, cheesy or putrid. Analyses indicated that during 7 days at 70 F the biacetyl content of control butter remained con-

stant but that more than one-half the biacetyl was destroyed in similar lots of butter contaminated with *Ps. putrefaciens*. The biacetyl content of butter prepared with culture and contaminated with *Ps. putrefaciens* remained relatively stable over a 7-day storage period at 70 F, although some destruction occurred. Elliker [111] reported that various bacteria, from widely differing genera, destroyed biacetyl added to sterile skim milk. Tests indicated that *Ps. putrefaciens* reduces biacetyl to acetylmethylcarbinol and 2,3-butylene glycol.

Wolochow *et al.*[112] considered that the chemical compounds imparting a sweaty-feet odor to skim milk cultures of *Ps. putrefaciens* are the same as those causing the odor in surface-taint butter. Dunkley *et al.*[113] concluded that acid mixtures obtained by steam distillation of acidified skim milk cultures of *Ps. putrefaciens* contain formic, acetic, butyric, and isovaleric acids, and that the substance causing the sweaty-feet odor of skim milk cultures of *Ps. putrefaciens* is isovaleric acid. Wolochow *et al.*[114] reported that surface taint did not develop in butter churned from cream containing *Ps. putrefaciens* cells that had been treated with such odorless bactericides as mercuric chloride; this indicates that living bacteria are necessary for development of the defect.

It would be expected that organisms other than *Ps. putrefaciens* could cause a putrid condition in butter. In agreement with this, Wolochow *et al.*[115] reported that a yellow pigmented organism, which they named *Flavobacterium maloloris,* produced a mild sweaty-feet odor in litmus milk and mild surface taint in butter. They believed it less important than *Ps. putrefaciens* as a cause of surface taint. These were the only 2 species suspected of surface taint-producing ability among 2,500 random isolations from normal and abnormal butter and creamery well water. Reynolds and Thornton [116] encountered only 2 cultures of *Fl. maloloris* among 589 isolations of yellow colony-producing bacteria from 140 samples of Alberta water. Studies on steam distillate residues of milk cultures of the organism indicated the same volatile acids as were reported for *Ps. putrefaciens.*

Tallowiness. Butter sometimes develops a flavor suggestive of tallow. The defect varies from a slight abnormality to an extreme condition in which the off flavor is pronounced and the butter may be bleached. It develops rather regularly in butter, if it is held sufficiently long, and butter showing various serious abnormalities, such as fishiness or cheesiness, eventually becomes tallowy. Appearance of talloviness in sterile butterfat suggests that direct action of organisms is not required.

When butter or butterfat is exposed to sunlight, it quickly becomes tallowy and bleached; diffuse light also accelerates production of the defect. Under practical conditions, temperature is an important factor in causing tallowiness. Butter held at 70 F commonly develops a tallowy flavor rapidly and, as the temperature of holding decreases, the period required for its appearance increases. Various lots of butter appear to differ in their susceptibility to the changes producing tallowiness, even when all the cream from which they were made was of fine quality.

Hunziker and Hosman [117] reported that butter in direct contact with either iron or copper developed a tallowy flavor and turned white; the action began where the butter was exposed to air and gradually went through the entire mass. Copper apparently caused a more rapid and more intense action than iron. In trials carried out by Palmer and Combs,[118] tallowiness was produced in butter by addition of 0.017% copper lactate to either raw or pasteurized cream. In each of three experiments, typical tallowiness and bleaching occurred in raw-cream butter before it appeared in the pasteurized-cream product. Addition of a small amount of copper salt to cream at the time of churning quite regularly results in rapid development of tallowiness in the butter. It occurs regardless of the *p*H of the butter serum.

Hussong and Hammer [119] reported various instances in which tallowiness and bleaching in butter were accompanied by high copper contents. In one instance there were blue areas on the butter before the bleaching began. Copper-contaminated wraps, which had areas of brown discoloration, apparently caused a high copper content in one lot of butter studied.

According to the findings of various investigators, production of tallowiness in milkfat involves an oxidation, presumably of the oleic acid. In the extreme condition there is a definite decrease in iodine number. Hunziker and Hosman [117] pointed out, however, that butter does not show a tallowy flavor when oleic acid, which has been exposed to the air until there has been a reduction in the iodine number, is added to it, but that butter containing free oleic acid rapidly becomes tallowy when exposed. Accordingly, these investigators consider that tallowiness is not the direct result of oxidized oleic acid, but is due to products formed in the butter during absorption of oxygen by oleic acid; since glycolic acid can be derived from oxidation of glycerol, esters of this compound are a possible cause of tallowiness. Trials showed that although addition of glycolic acid did not give

butter a tallowy flavor, glycolic acid esters of the fatty acids of butter produced a tallowy flavor when warmed with butterfat, especially the glycolic acid ester of oleic acid. The tallowiness was produced in the absence of air. Comparison samples, in which oleic acid alone was added, showed no tallowiness.

King [120] noted that, in the presence of air, butterfat reacted with biacetyl and became tallowy and bleached. The reaction was accelerated by light. King suggested that biacetyl may act on butterfat by oxidizing oleic acid to oleic acid peroxide which then breaks up into various compounds having a tallowy flavor, the biacetyl being reduced to the carbinol or glycol. In butter only a relatively small amount of the biacetyl is in the fat, and the opportunity for reactions between fat and biacetyl is less than when biacetyl is added to butterfat.

Reinart [121] reported that passing a stream of air for 2 hr through fresh cream, at 131 F, to which 0.02, 0.5, and 2% biacetyl had been added increased the peroxide values over the controls with no added biacetyl. Addition of biacetyl to butter caused a similar increase in peroxide value after 20 days in the dark at room temperature; these samples, however, had been selected from dairies known to produce butter with oxidative effects. Destruction of carotene and tocopherol increased with increasing amounts of biacetyl. Bleaching also took place.

Wiley [122] found that in butter, low *p*H, presence of culture organisms, low pasteurization temperatures, and salt all separately favored oxidation of fat at cold storage temperatures. Oxidation in the presence of culture organisms, even when the acid formed by them was neutralized before churning, indicated a fat-oxidizing system in ripened cream. This could be some product of bacterial metabolism acting as a pro-oxidant but it was not biacetyl or acetylmethylcarbinol. Wiley noted the good keeping quality of butter made from ripened cream which had been neutralized and then pasteurized and reported that such butter was no more markedly oxidized during cold storage than sweet-cream control butter. This suggests that if a pro-oxidant is formed during ripening it is destroyed by pasteurization.

According to Hietaranta,[123] microbial action in butter prevents, or perceptibly retards, fat oxidation due to reducing the oxidation-reduction potential. Another effect can be brought about by proteolytic bacteria; in butter with less acidity, the proteolytic enzymes of bacteria are activated and protein decomposition products can bind copper and iron ions and reduce their catalytic effect. Organisms pro-

ducing catalase may prevent continued oxidation by decomposing fat peroxides.

Preventing oxidation in butter is one of the most important problems in the butter industry. Tallowiness in a lot of butter continues to get worse over long periods. Apparently, no simple procedure prevents it. Butter made with or without culture becomes tallowy. Various antioxidants have been suggested for use in butter and sometimes may delay appearance of the defect but do not prevent it. There also may be some delay as a result of high heat treatment of the cream, and this general procedure may prove to be especially useful. Elimination of metal contamination definitely is helpful in delaying the defect.

Additional Defects Due to Organisms. Chemical changes produce only a limited number of types of flavor defects in butter. Action of micro-organisms, however, produces a variety of types.

Organisms of the *Escherichia-Aerobacter* group sometimes produce unclean flavors in butter. The *Aerobacter* species are more important in this respect than the *Escherichia* species. Relatively low temperatures and also salt have striking effects in restraining the organisms.[124]

An ester-like flavor sometimes appears in butter. Apparently, it can be produced by various organisms. When *Ps. fragi* is growing in butter an ester-like flavor often is noted before rancidity develops.

A skunk-like odor that appeared in 3 samples of unsalted butter from one plant when they were held at 70 F to test the keeping quality was studied by Claydon and Hammer.[125] The defect was reproduced by churning pasteurized sweet cream inoculated with the defective butter. The causative organism was isolated and named *Pseudomonas mephitica.* On inoculating pasteurized cream and churning, the defect developed in unsalted butter made from cream having *p*H values from 5.0 to 7.5. Salt had an inhibitory effect on production of the defect, but thorough working was necessary to prevent its appearance. The skunk-like odor was produced in unsalted butter by inoculating small amounts of a culture into the water used for washing.

Experience of butter plants indicates that a skunk-like odor sometimes is produced in butter as a result of the consumption of one or more pasture plants by the cows. Olson *et al.*[126] reported this flavor in milk from cows which have consumed wild carrots (*Spermolepis echinata*) and mules tail (*Erigeron canadensis*); when either weed is consumed separately, the off flavor is slight or nondetectable but, if they are consumed together, the off flavor generally is distinct. Re-

moval of cows from pastures having these weeds 2 to 4 hr before milking does not prevent the carry-through of the flavor into butter made from the milk.

Certain lots of butter have a flavor suggestive of roquefort cheese. Presumably *Penicillium roqueforti* and related forms are involved primarily. The probable cause of the flavor is the production of methyl ketones, especially methyl-*n*-amyl ketone.[127] The defect may be noted when there is no macroscopic mold growth. In rare instances it appears that a yeasty flavor has been produced in butter through growth of lactose-fermenting yeasts in it.

With many species of organisms capable of growth in butter under favorable conditions, a variety of flavors would be expected. Many of them are inconspicuous and indefinite and of relatively little commercial importance. Often it is difficult to isolate the causative species and the defects actually may be due to associative action.

Sectional Contamination. Jensen[128] reported on sectional contamination of butter in which localized areas take on a distinct off flavor so that there are variations in quality in different portions of the lot. The butter commonly shows zones of tainted product separated by zones of normal product. The defect does not occur in fresh butter but at 40 to 50 F, or higher, it may develop rapidly, sometimes becoming distinct in 3 to 4 days. The off flavors encountered in the affected areas vary from staleness to distinct fruitiness, cheesiness, and rancidity. Observations indicated that the defective areas in a lot of butter showing sectional contamination tend to follow the junctions of the several layers of butter going to make up a block of hand-packed product. The defect was attributed to localized action of organisms added to the butter by water and utensils used in packing operations. The primary source of the causal organisms was the water supply; a secondary source was the wood of the utensils.

Control of Microbiological Defects. Complete control of the flavor defects of butter due to growth of micro-organisms requires attention to many details. In general, the species involved are destroyed by adequate pasteurization so their presence in the raw cream presents no problem. Following the pasteurization, contamination of the cream and butter must be avoided, and this requires attention to the various sources from which organisms may come.

The restraining effects of physical condition, salt content, holding temperature, and acidity on growth of organisms in butter frequently prevents spoilage with insanitary plant methods which would be expected to cause it. This may lead to more and more carelessness in

production procedures until eventually spoilage occurs. Such an outbreak may be difficult to control immediately.

In some instances spoilage outbreaks develop in plants in which the operators believe they are using adequate precautions and then it is difficult to locate the sources of contamination. Under these conditions, wood churns that, because of their age and mechanical state, are difficult to sanitize, leaky vat liners, and contaminated water supplies appear to be at fault most frequently.

COLOR CHANGES IN BUTTER DUE TO ORGANISMS

Color changes sometimes occur in butter as a result of growth of organisms. Various molds are especially important in this connection. The color changes do not take place rapidly; they occur more readily in unsalted than in salted butter.

Moldy Butter. Development of molds occurs with both print and bulk butter. Growth is confined largely to portions at or near the surface, owing to the oxygen requirements of the organisms. Mold on butter not only influences the appearance of the product but also may affect the flavor, and this can occur before growth is evident macroscopically. In general, moldy butter is an indication of unsatisfactory methods of manufacturing and/or packing. However, various factors, such as high holding temperatures, may be the immediate cause of the condition; unsatisfactory refrigeration repeatedly has resulted in moldy butter reaching the markets.

Types of Molding. The moldy areas that develop on butter vary widely in appearance, owing to differences in the species involved and also in the extent of growth. In some instances two or even more molds may be growing in combination.

Thom and Shaw [129] recognized three main types of mold growth: (1) A common greenish growth on the butter and sometimes on the wraps, liners, etc., which usually is due to a *Penicillium* species; (2) a smudged type which suggests soot or dirty finger marks on the butter and is due to *Alternaria* species; and (3) orange or yellow discolorations, with little or no surface growth, which appear to be caused by *G. candidum*.

Sources of Molds in Butter. Molds in butter can come from various sources, as indicated by the general sources of organisms in the product. In field studies on the sources of molds in Minnesota creameries, Macy and Combs [130] found that the raw cream was heavily contaminated, even when most of it was sweet and fresh, and that

dry parchment, piping, pumps, churns, water, culture, and salt sometimes were contaminated. Hood and White [131] noted that the interior of moldy butter contained large numbers of yeasts and molds, indicating that the butter must have been heavily contaminated.

Preventing Contamination with Molds. Production of cream free of molds is impossible under practical conditions, but proper pasteurization controls the contamination of butter from this source. The studies of Thom and Ayers [132] show the general resistance of mold spores to heat. When heated for 30 min in milk, a number of species were killed at 130 F whereas a few were destroyed at a lower temperature; several species survived 140 F, but 145 F destroyed mold spores completely enough to render them a negligible factor in the future changes in milk. With a heating period of 30 sec, 145 F killed very few species, 155 F killed most species, whereas 165 to 175 F killed practically all although some species survived occasionally. Dry heat was much less effective.

Preventing contamination of pasteurized cream and butter with molds involves attention to the various sources from which organisms can gain entrance to these products. The equipment, particularly the churns, often has added many molds to cream and butter. Contamination from the air should be limited as much as possible by exposing the cream and butter to the air for the minimum period.

Riddet and Neill [133] noted that molds originating in butter boxes may infect the surface layers of butter packed in them. A double layer of 28/30 lb parchment did not prevent mold mycelia from penetrating the surface of butter from the inner surface of the box, but aluminum-foil parchment did prevent it. Immersion of butter box timber for 10 min in a 0.1% aqueous solution of sodium salicylanilide appeared to be an effective method of preventing mold growth on butter boxes.

Growth of Molds in Butter. Contamination of butter with mold is not necessarily followed by growth, and many lots of butter showing excessive mold counts have a normal appearance for extended periods. More or less definite requirements must be met before growth occurs.

Molds grow on a variety of materials, many of which are comparatively resistant to action of organisms, so that the nutrients supplied by butter undoubtedly are satisfactory. The fat is less suitable for growth than the curd, and some of the butter constituents that are present in very small amounts may be especially important in this connection. The salt present in butter has an inhibiting effect on

mold development, as is shown by the fact that mold is more troublesome with unsalted butter than with salted. Early studies of Gripenberg [134] recognized the importance of salt in controlling mold growth and all subsequent studies have emphasized it. Thom and Shaw [129] concluded that 2.5 to 3% salt in butter, which is equivalent to 12 to 15% brine, is sufficient to eliminate mold or reduce it to a negligible amount. However, investigations showed that some lots of butter containing these amounts reached the markets in a moldy condition. Hastings [135] noted that amounts of salt which effectively prevent germination of mold spores do not stop growth of vegetative cells; accordingly, if pieces of mold filaments are present in or on butter, as a result of contamination from some source, they may continue their development in the presence of considerable salt, although salt may decrease the rate of growth.

Figure 40. Mold development on ¼ lb prints of butter.

Temperature has a very direct influence on the development of molds on butter. In general, growth is progressively reduced as the temperature is decreased from room temperature, until at 32 F it is very slow if it occurs at all. Under practical conditions rapid development of molds takes place principally when the butter is held at temperatures that are comparatively high.

High humidity greatly favors growth of molds. Moisture content of the air appears to be more important than that of the material on which the molds are growing and, with a favorable humidity, molds may develop on nutrients very low in water. Thom and Shaw [129] found that molds would not grow on butter exposed to humidities of 70% or lower, whereas a species of *Penicillium* grew on a mixture of butterfat and skim milk powder held at a favorable humidity. Air spaces in butter packages have an opportunity to develop high humidities because the air in them is more or less confined and can take up water from the butter and moist packing materials.

Because free oxygen is required, mold growth in butter is confined to portions where air is available. Occasionally, growth penetrates several millimeters from a surface in contact with an oxygen supply. Cracks left in butter by careless packing may be a factor in mold development because the air supply which they provide favors both

Figure 41. Effect of mold inhibitor on butter wraps. *Left:* control. *Right:* wrapped in parchment treated with 10% solution of calcium propionate.

initial mold growth and extension of that already begun. Butter, especially if it is leaky, tends to collect water next to the wrap or liner and, when this drains away, air spaces of considerable size may result.

Mold Inhibitors. Various chemicals have been suggested for control of mold growth on butter and on parchment in contact with it. Propionates have received special consideration in this connection because of their effect in controlling mold growth on bread.

Ingle [136] reported tests on unsalted butter stored at 60 F and 100% humidity which indicated that wraps containing either 6% calcium propionate or 12% sodium propionate greatly inhibit mold growth on butter. Wetting propionate-impregnated wraps before wrapping the

butter gave better results than using the impregnated wraps dry. There seemed to be little difference in action between parchments treated with sodium and calcium propionates. Olson and Macy [137] found that parchment paper treated in 5% calcium propionate solution acidified to *p*H 5.5 with lactic acid was fully as effective in inhibiting mold growth on unsalted butter as parchments treated in 10% unacidified solution. Solutions of either calcium or sodium propionate were superior to a saturated sodium chloride solution for inhibition of surface mold growth. Calcium propionate-impregnated parchments were effective with dry wrapping of butter but immersing such paper in water before the wrapping leached the propionate from the paper. Heating propionate solutions did not impair their effectiveness. The *Penicillium* species used was markedly more resistant to the inhibiting effect of propionate than species of other genera. Undissociated propionic acid appeared to be mainly responsible for the inhibitory effect.[138]

Propionates also have an effect on certain bacteria. Mann and Frazier [139] showed that sodium and calcium propionates readily inhibited development of *E. coli* and *Alc. viscosus* and exercised considerable restraint on *S. lactis,* but *S. thermophilus* and *L. bulgaricus* were more resistant; there was comparatively little effect on *G. candidum.* Wolford and Andersen [140] investigated the action of propionates in controlling microbial growth in fruits and vegetables and found the inhibitory action of sodium propionate on micro-organisms varied with *p*H of the substrate, being greater at low *p*H values. Concentrations which destroy some organisms at *p*H 3.5 to 4.5 may not retard their growth at *p*H 6 to 7.

There should be little objection to use of propionates in connection with food products because of their production, through fermentation, in various dairy products, particularly swiss-type cheese. When Harshbarger [141] fed calcium or sodium propionate to growing white rats in quantities up to 3% of the diet, there was no decrease in the gain in weight.

The fatty acids offer some interesting possibilities in connection with inhibition of molds on various products.[142, 143] Butyric acid appears to have good fungistatic properties and the same is true of certain other acids. The objectionable flavors of some of the acids higher than propionic is of importance in the case of food products; also, their low solubilities in water may interfere with their efficient use.

Other agents also have been suggested as mold inhibitors. Wolf [144] inoculated unsalted butter with spores of *Penicillium expansum* and

found that wraps prepared with dehydroacetic acid added to the wax coating inhibited mold growth in contrast with untreated controls. Brunner and Mallmann [145] wrapped butter in untreated parchment, in wet or dry parchment impregnated with sodium dehydroacetate, or in wet parchment impregnated and dipped in 1% solution of the salt. The untreated samples showed surface mold after 14 days, the dipped samples showed little or no mold growth after 56 days, and the other two classes were intermediate.

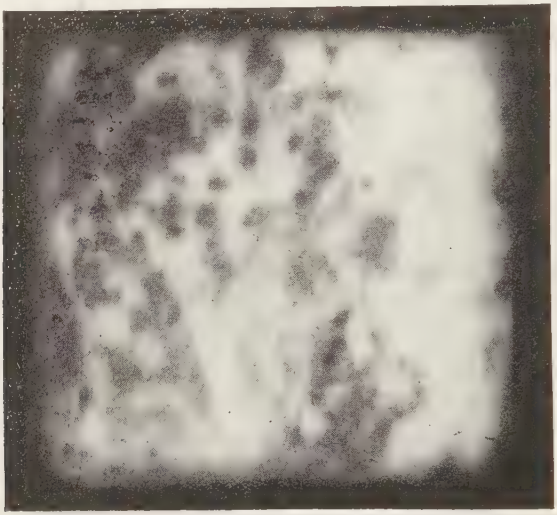


Figure 42. Surface discoloration of butter due to *Pseudomonas nigrifaciens*. Courtesy A. H. White.

Color Changes Due to Pink Yeasts. In some few instances pink yeasts have been responsible for a color change in butter. It appears either as a pink tinge over a considerable area or as small, sharply defined spots. At least some of the pink yeasts develop better in unsalted than in salted butter.

Bacterial Surface Discoloration. In Great Britain [146] Hiscox reported surface discoloration of mildly salted butter that was cut into pats and held in storage awaiting distribution. Two strains of bacteria producing bluish-black pigment were isolated from discolored areas.

Samples of Canadian print butter showing black to reddish-brown discoloration in irregular patches on the surface were investigated by White.[147] The discoloration penetrated to a depth of about one-

eighth in. Microscopic preparations made from discolored portions revealed many small gram-negative rods. An organism capable of causing the defect was isolated by plating the butter on nutrient agar with 1.5% sodium chloride added or by streaking on slopes of the same medium, incubation being at 39.2 or 59 F. It produced blackish, insoluble pigment on culture media with low concentrations of salt; optimum conditions for pigmentation appeared to be 39.2 F with 1.5 to 2.5% salt. The defect was produced in butter by inoculating the cream used for churning and also by inoculating the butter directly, either with infected wash water or during the working process, provided 0.5% salt was added to give about 3% salt in the serum. Low concentrations of salt appeared to be essential for proper growth and characteristic pigmentation by the organism but, with over 5% salt in broth culture media or butter serum, pigmentation was inhibited and growth retarded. The organism was named *Pseudomonas nigrifaciens.*

Leggatt [148] noted that bacterial discoloration of butter usually is caused by *Ps. nigrifaciens* and reported that in the four cases investigated the sources of the causative organism were water supply (2), butter boxes (1), and salt solution (0.5%) used to soak butter wraps (1). Two of the cases studied involved butter containing 2% salt.

Butter showing discoloration through growth of *Ps. nigrifaciens* has been encountered in various sections of the United States. Commonly, *Ps. nigrifaciens* can be isolated by plating on a salt medium. On one occasion the defect was encountered in putrid butter and both *Ps. nigrifaciens* and *Ps. putrefaciens* were isolated.

Color Changes Not Due to Organisms. Color changes have been noted in butter due to causes other than action of organisms.

Pink Color Due to Sulfur Dioxide. Hammer and Olson[149] studied print butter, from two widely separated sources, that showed areas of distinct pink color at the surface. Investigations indicated the butter contained neither unusual numbers nor types of organisms. When portions of the butter that were normal in appearance were exposed to sulfur dioxide, a pink color quickly developed. The butter from each source had been held in a refrigerator using sulfur dioxide as a refrigerant and, in the one instance in which an examination was made, a leak permitted the gas to escape into the food compartment. Butter from each source developed a pink color when exposed to various reagents, including a number of acids. The butter was made with certified color. Experimental butter colored with either yellow AB or yellow OB developed a pink color when exposed to various reagents.

Surface Darkening. The surface of butter sometimes shows a

darkening of the normal yellow color that is especially noticeable when the surface color is compared with the color of the interior. The defect occurs most often in prints, particularly when there has been an opportunity for drying, and is considered to be due to loss of moisture from the surface layers. In New Zealand, Barnicoat [150] noted that cold-storage butter often assumes a deep tint that is described as "primrose." It is not due to freezing since fresh butter at room temperature can develop the defect. Barnicoat concluded that the color is due to a surface desiccation which alters the structure and appearance of the butter, just as the opposite effect may be observed during its manufacture, when incorporation of water and working cause the butter to assume a lighter color. For control, he suggested use of a wrap impermeable to moisture and also effective sealing.

PRODUCING CREAM FOR BUTTER

On many farms the milk is skimmed and the skim milk used for feed. At regular intervals or when sufficient cream has accumulated it is sold, usually for butter making purposes. Some farm-skimmed cream is sweet when marketed, but much of it is sour. Cream for butter also is obtained from various dairy plant operations.

In general, milk to be skimmed on farms is produced with less care than that subject to inspection and perhaps other regulations at plants. However, purchasers of farm-separated cream normally have standards for an acceptable product and reject cream considered to be unsuited to human consumption. In turn, butter plants are supervised by regulatory officials who may seize butter which is considered to be made from unfit cream.

Farm Separation of Cream. On most farms the cream is obtained by centrifugal separation but, with only a small volume of milk available, other methods may be employed.

Centrifugal Separation. The preferred method of obtaining cream is with a centrifugal separator. It carries out the process quickly so that the cream can be cooled soon after the milk is drawn (see p. 197). If separators are not washed every time they are used, milk and separator slime left in them afford an ideal food supply for bacteria so that, at suitable temperatures, growth is rapid. When the machines then are operated, very large numbers of organisms and undesirable odors may be added to the skim milk and cream, especially at the beginning of the operation.

Gravity Separation. Cream obtained with gravity separation often has a high bacterial count. In 6 trials with such separation, Anderson [151] found the whole milk averaged 2,708,000 bacteria per ml, the skim milk 2,050,000, and the cream 15,416,000. Leete [152] carried out gravity separation in a can 18 in. high and 8.5 in. in diameter, which was carefully washed and steamed before use. During the separation the can was kept in a tank of ice and water. In 25 runs the average bacterial counts per milliliter were: Whole milk 135,880, skim milk 33,556, and cream 283,680.

In gravity separation the slow movement of the cream to the surface apparently provides suitable conditions for many bacteria to be entangled in the clusters of fat globules. Unless low temperatures can be provided, the time required affords opportunity for growth of organisms. Extraneous matter in the milk also may be carried into the cream.

Water Dilution Separation. Milk sometimes is diluted with water, the mixture held for a time, and the cream then removed. In general, this procedure yields poor-quality cream. Often the water contains organisms that bring about rapid deterioration of the cream, it may have undesirable flavors, and it may contain extraneous matter, some of which is carried into the cream. Use of low temperatures helps limit the growth of organisms during the separation. Edmondson and Stallcup [153] found that the quality of cream from a centrifugal separator, as measured by flavor and mold content, was considerably higher than that from a water dilution separator. In all cases butter from the centrifugal separated cream was higher in score.

Holding Cream on Farms. Cream that is held on farms should be cooled rapidly and held at low temperatures. With large volumes of cold water the problem is simplified and, if deliveries are frequent, sweet cream can be marketed. In many areas there is no such supply of water and deliveries are infrequent so that souring is inevitable. With poor holding facilities, frequent cream deliveries are needed if the sour product is to be satisfactory.

On some farms mechanical refrigeration is used for holding cream. Such holding should not be greatly prolonged; although acid production may be very slow, psychrophiles that attack protein and/or fat may grow and milk lipase may be active.

Price *et al.*[154] reported that cream cooled and held in a tank filled with water at 60 F, the water being changed twice daily, remained sweet for 18 hr but was sour after 42 hr with an acidity of 0.4 to 0.5%; with the water at 50 F, the cream had an acidity of 0.20 after 42 hr

and of 0.41 after 66 hr. Cream held in an ordinary ice refrigerator at 45 F cooled more slowly for the first 7 hr than when held in water at 50 F; it had an acidity of 0.19% after 42 hr and of 0.23 after 66 hr. Cream cooled in an electric refrigerator having dry storage remained sweet for twice-weekly shipments, both with natural and forced air circulation. Cream held in refrigerated water at 35 to 40 F dropped from 80 to 56 F in 1 hr and remained sweet and of good quality for 90 hr.

Care of Cream in Plants and Stations. Unless cream received at butter plants is to be pasteurized very soon, it must be cooled (unless already cold) and then held cold if rapid deterioration is to be avoided. In vats of uncooled sour cream held overnight during warm weather, growth of *G. candidum* is surprisingly rapid, and there may be a film of mold growth over the entire surface by morning. Undoubtedly, various other species also grow actively although not so conspicuously.

Much of the sour cream produced on farms goes to cream stations from which it is shipped to butter plants. In the stations also the cream should be cooled at once, held cold, and then shipped frequently. Use of mechanical refrigeration in certain stations to cool and hold the cream has enabled them to deliver much better cream to the plants. Cans of cream that are well cooled warm only slowly during shipment which helps avoid serious flavor deterioration.

Evaporation and Spray Systems of Holding Cream. When better facilities are lacking, an evaporation system in which water is evaporated from wet sacks or other wicking material surrounding the cans, or a spray method in which water is sprayed over the cans, sometimes has been used with cream. Under suitable conditions, the methods may reduce the temperature of the cream somewhat,[155] but, in general, they are inadequate for the proper holding of cream.

TESTS ON CREAM AND BUTTER

General Quality of Cream

The general quality of the cream used is the most important factor determining the flavor of butter. Various tests have been suggested for evaluating it, but examination for flavor and appearance is the most widely used. Many butter plants make more than one grade of butter by dividing the lots of cream into two or more classes on the basis of flavor and appearance and also reject certain lots as unfit for use. In receiving cream at a plant, an efficient operation requires that

the various lots be classified rapidly and an examination for flavor and appearance meets this requirement.

Mold Hyphae in Cream and Butter

The relative abundance of mold hyphae in butter has been employed in attempts to detect the use of unfit cream in butter manufacture and, accordingly, the hyphae in cream and butter have been studied extensively.

Molds in Cream. Following preliminary work by Wildman,[156] a method for the visual demonstration of mold in cream was devised by Parsons.[157] It consists of flocculating the mold hyphae in 9 ml of cream with heat and a special reagent and filtering them on an organdy disc. The preparation thus obtained is compared with standards and classified. The method is rapid and gives results that are very satisfactory for determining the relative abundance of mold in different lots of cream. Some bacteria and yeasts commonly are flocculated with the mold hyphae.

Sweet cream contains very few mold hyphae. Sour cream contains variable numbers, depending largely on the conditions of holding. As ordinarily produced, cream contains molds and, if it is held under suitable conditions, growth will occur.

Various species of molds grow on cream but, in general, *G. candidum* is the most important. The film and later the wrinkled membrane produced when it grows extensively on cream are very conspicuous and characteristic. If the conditions of holding permit rapid development of organisms, other types of surface growth sometimes occur; these may be due to molds, yeasts, or bacteria, extensive growth of certain species of bacteria being made possible by the action of molds in increasing the *p*H.

Mold growth on unagitated cream is primarily at the surface and the top 0.5 in. of a lot of cream contains most of the mold, with the lower layers showing little. Because of this, the relationship between surface area and volume of a lot of cream [158] is an important factor in determining its mold content. Cream in a shallow layer may contain a relatively large amount of mold when mixed and tested, whereas an equal volume of cream from the same original lot but held in a much deeper layer may show comparatively little.

Temperature and period of holding are important factors in determining mold growth on cream. Growth is rapid over a rather wide temperature range. There may be variation from one lot of cream to another because of differences in the species of molds present, but

ordinarily growth is active from 70 to 85 F.[159, 160, 161] With some lots of cream growth is quite rapid at 60 F, but commonly it is very slow at 50 F. Above 95 F growth often is slow, if it occurs at all, because such temperatures definitely are too high for most species.[160, 161] At all temperatures permitting mold growth, it continues over a long period.

Extent of the contamination with molds obviously is a factor in their growth on cream, but the variations in growth rates under different holding conditions easily outweigh variations in extent of the original contamination. Mold growth on heavily contaminated cream

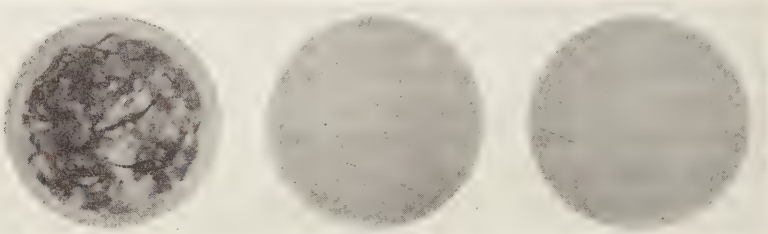


Figure 43. Mold at different levels in undisturbed cream (Parsons visual mold test). *Left:* top 0.5 in.; *middle:* second 0.5 in.; *right:* second 1 in.

is controlled readily by low temperatures, and it can be extensive on lightly contaminated cream held at favorable temperatures.

Relatively large shipments of cream tend to have lower mold contents than relatively small shipments;[161, 162] various factors may influence this, such as surface-volume relationships, care in production, age, etc. High-fat cream tends to have less mold than low-fat cream.[158, 161, 163] Excessive acidities limit mold growth.[160, 162] Rancid cream tends to be relatively low in mold,[161] presumably because of the mold-inhibiting properties of certain fatty acids. The humidity under which the cream is held apparently does not affect mold growth on it.[161]

Elliker and Brown [164] found that, when cream was stored at 60 or 80 F for 4 or 7 days, centrifugal separation resulted in lower mold contents than did water-dilution or shallow-pan separation. With fresh milk containing a comparatively large number of molds, centrifugal separation removed approximately 90% from both the cream and skim milk. Water-dilution and shallow-pan separation tended to concentrate molds in the cream.

Various schemes of stirring or layering have been suggested to keep

down mold growth on cream. The results obtained in different studies are not in agreement,[158, 161, 162, 165] presumably because of variations in the procedures followed. Frequent stirring, for example, twice a day, tends to keep down mold growth but does not control it; stirring once a day may have little effect or may tend to increase it. Attempts to add new portions of cream to an accumulated lot by pouring the new portions so that they will float on the accumulated lot ordinarily have not kept down the mold content of cream; often there is a tendency for the mold growth to float to the new surface.

Molds in Butter. Wildman [156] determined the relative abundance of mold hyphae in different lots of butter. The method involves microscopic examination of the product under specified conditions,[1] with the reporting of the results on the basis of the percentage of positive fields.

Ordinarily, the mold hyphae seen in examining butter are dead as a result of pasteurization of the cream, and a product containing many hyphae may show negligible numbers of colonies on culture. Butter with a high hyphae content normally comes from cream containing a large amount of mold growth. Poorly cleaned equipment, etc., are other possible sources of mold hyphae in butter.

The Parsons [157] procedure for visual demonstration of mold in cream can be applied to butter and comparisons of mold contents of different lots of butter made very satisfactorily. With it, examinations are made rapidly and, by developing a set of standards, the results can be related rather closely to the results of the standard procedure.

Butter made from sweet cream regularly has a very low hyphae content. Butter made from sour cream may have a low, moderate, or high content, depending largely on the conditions under which the cream is held, although extent of the contamination also may be a factor.

Retention in Butter of Hyphae in Cream. Adams and Parfitt [158] found that, of the molds in cream, $<10\%$ to as high as 70% were retained in the butter and that in 66.2% of 121 comparisons the retention was below 31%. Size of butter granules and degree of neutralization of the cream did not affect the retention. Elliker [166] reported that butter usually retained $>50\%$ of the total length of mold filaments in cream and that the wash water contained a very small percentage. During churning the butter appeared to retain the long filaments, whereas most of the tiny fragments were in the buttermilk.

Comparison of Flavor Grade and Mold Content of Butter. Rapid growth of molds in cream commonly is accompanied by growth of other organisms and by flavor deterioration so there is a general rela-

tionship between mold content of cream and its organoleptic quality. However, it is only general because factors other than mold growth are involved in flavor deterioration of cream. As a result, some butter scoring relatively high for a sour cream product may have high hyphae contents and some butter scoring very low may have low contents.

Various investigators have noted the lack of correlation between flavor grade and mold content of butter. For example, Elliker and Horrall [167] studied 1,385 samples of butter and found that the mold mycelia values did not correlate closely with organoleptic grades over the various seasons. The values showed the expected pronounced seasonal variation, with higher mold contents in the warm months. Organoleptic grades appeared fairly uniform throughout the seasons, although slightly more poor-quality butter appeared to be made in the fall. The authors considered that, in actual quality of butter in the area studied, greater improvement might be made if more attention was paid to organoleptic grade of cream and less to mold content.

Certain areas apparently produce butter with a generally higher hyphae value than other areas supplying butter of the same general quality. For the most part, northern states with shorter summers and cooler well water have lower hyphae values than southern states, even with butter of essentially the same quality. Again, butter from the southeastern states seems to be higher in hyphae than butter of comparable quality from the southwestern states. In certain areas, winter butter has much lower hyphae values than summer butter of the same quality.

Molds are not the main cause of flavor deterioration in cream so no close correlation between mold content and flavor grade of butter can be expected. Some lots of cream having a very poor flavor contain little mold, and other lots having a much better flavor contain relatively large amounts. Mold growth is primarily at the surface of cream while deterioration occurs throughout the mass. Many lots of very high acid cream, which are certain to make low-grade butter, contain relatively little mold. Comparisons show that flavor deterioration in cream is much more rapid than the increase in mold content.

Different investigators have noted that cream held in buying stations may deteriorate rapidly in flavor without a significant change in mold content. For example, Morrison *et al.*[168] studied 113 lots of cream held in 10-gal cans in buying stations for 1 or 2 days at slightly below atmospheric temperature. The quality of cream, as indicated by grade and acidity, deteriorated rapidly while frequently the visual mold test did not reflect this deterioration.

In trials by Adams and Parfitt,[158] controlling the mold growth on a portion of a lot of cream by means of a layer of mineral oil did not result in a significant effect on the flavor of the butter made from it compared to that of butter made from the cream on which mold growth occurred. Elliker [159] limited the growth of molds in cream by twice-a-day stirring but found that the flavor of the unstirred cream was unquestionably superior to that of the stirred cream; he suggested this may have been due to the larger numbers of yeasts and higher titratable acidities in the stirred cream.

Water-insoluble Acids (WIA) in Cream and Butter

The fat in cream may be hydrolyzed by lipolytic micro-organisms or milk lipase with the liberation of fatty acids, including water insoluble acids (WIA). When such cream is churned, the butter has a higher content of WIA than without fat hydrolysis in the cream. Regulatory officials have used the WIA content of cream and butter to detect decomposition in the products. Results are expressed as the milligrams WIA per 100 g fat.

WIA in Cream. The method for determining WIA in cream and butter was outlined by Hillig,[169] who conducted experiments involving the relation between WIA and cream decomposition. The results indicate that, as cream ages, the fat frequently is hydrolyzed and WIA are formed; in some cases the WIA content is far in excess of that of sweet cream. Butter manufactured from cream showing no decomposition contained less WIA than butter manufactured from decomposed cream. Freeman and Barkman [170] noted that the WIA content of cream increased with age, but the increase was not consistent under all conditions. Cream produced in the fall and winter showed a more definite relation between age and WIA content than cream produced in spring and summer. Organoleptic grading of cream was not entirely satisfactory as a means of segregating cream into classes corresponding to the WIA contents of the resulting butter.

When sterilized cream is inoculated with a culture of a lipolytic micro-organism, there is a definite relation between time and temperature of incubation and WIA content. However, with raw cream, the results are somewhat variable. Babel [171] found practically no difference in WIA contents of a split lot of raw cream held at 55 and 75 F for 1 to 10 days, which indicates that, when cream sours rapidly, fat hydrolysis is delayed by the low *p*H. Peters *et al.*[172] obtained marked increases in the WIA contents of most raw cream samples held at 38 F, but samples held at 60, 72, and 85 F gave variable results, both

high and low WIA values being obtained. Purko *et al.*[173] inoculated sterile cream with *G. candidum* and held it at 39.2, 50, 60.8, 69.8, 84.2 and 98.6 F for 3 days; after incubation the cream had WIA values of 229, 514, 2,508, 5,826, 7,626, and 11,057, respectively. Parmelee and Babel [174] studied the effect of holding raw cream at 40, 55, 70 and 85 F for 4 and 7 days. One lot had higher WIA contents with holding at the higher temperatures, whereas another lot had the highest value at 40 F after holding 4 days and at 85 F after holding 7 days.

Hillig and North [175] held fresh cream at 39.2 F with and without addition of 0.1% formaldehyde. Little or no increase in WIA occurred with formaldehyde, and cream without formaldehyde did not show a pronounced increase in WIA until it had deteriorated to a point where organoleptic classification condemned it for use in butter manufacture. Addition of about 0.2% formaldehyde to cream [172] decreased the rate of formation of WIA but permitted some increase; the increase was attributed to milk lipase. Other preservatives have been studied in trials involving WIA determinations on cream.[174] Sodium fluoride (0.1%) did not prevent microbial growth and chloral hydrate (0.2%) interfered with enzyme activity. Sucrose, added at the rate of twice the moisture content of the cream, effectively limited growth of micro-organisms and cream containing it had the highest WIA content, presumably due to milk lipase.

WIA in Butter. Twelve samples of commercial butter tested by Hillig [176] had WIA values of 51 to 1,278. Molecular weights of the acids indicated they were primarily oleic and palmitic acids. Analyses made later [177] on 321 samples gave values from 21 to 3,299. Armstrong and Harper [178] analyzed 32 samples of butter and obtained WIA values from 128 to 612. There was a general relationship between flavor score and WIA content, but many inconsistencies were noted. The relationship was more consistent with samples scoring over 90 than with those scoring 90 or less. The WIA contents ranged from 127 to 303 for 90-score butter, from 174 to 409 for 89-score, and from 183 to 612 for 88-score. Feder and Breazeale [179] tested 368 samples of butter for WIA, the samples being selected to contain a high proportion of low-grade butter. Only 11 of the samples had WIA values over 400. Commercial butter from 4 plants in Indiana [180] contained more WIA in the winter and spring than in the summer and fall. Thirty-four samples of butter from 3 Indiana plants [174] were tested for WIA over a period of 1 yr; 18 of the samples classed as 90-score contained 89 to 575 mg WIA, 12 samples classed as 89-score contained 179 to 707 mg, and 4 samples classed as "no grade" contained

107 to 323 mg. It was noted that the flavor score of butter is assessed by an organoleptic examination which takes into account all types of deterioration so there is little reason to expect a close correlation between WIA content and flavor score.

The storage temperatures commonly used for commercial butter permit little or no microbial growth or lipase activity in it. Hillig and Ahlmann [181] found no significant change in the WIA content of butter held at 40 F for 75 days or at 0 F for 5 months. Kester *et al.*[182] noted that salted (2%) butter made from cream inoculated with *Ps. fragi* showed little change in WIA when held 30 days at 48 F although a similar unsalted sample held under the same conditions showed a marked increase. One strain of *Ps. fluorescens* had little or no effect on the WIA content of butter held at 48 F whereas another strain gave a slight increase. *Ach. lipolyticum* increased the WIA content of both salted and unsalted butter held at 48 F for 30 days; the increase was greater in the unsalted product.

Factors Influencing WIA Content of Cream and Butter. In addition to the action of lipolytic micro-organisms and milk enzymes, certain other factors may influence the WIA values obtained on cream and butter.

The Method. Investigation [180] of the procedure for determination of WIA indicated that, for certain samples, the results were influenced by the amount of alkali used for neutralizing the ether-water mixture of butter; with an increase in alkali, the WIA was increased. In other trials [183] the effect of alkali was considered insignificant, even though some butter samples showed an increase in WIA by use of more alkali.

Milk. Butter prepared from the fresh milk of individual cows had WIA values from 30 to 550, according to the results of Parmelee and Babel.[174] Of 36 milk samples used for butter, 10 gave WIA values greater than 100. The high values were obtained on milk from cows fed dry feed. Hillig and Palmer,[184] using butter prepared from a herd of cows on pasture and later from the same animals on dry feed, concluded that feed did not influence the WIA values. Fresh butter prepared from fresh milk had WIA values ranging from 55 to 173.

Water Separators. Water separators tend to decrease the lactose content of cream and the water used may add lipolytic micro-organisms to the product. Hillig *et al.*[177] considered that use of water separators was one of the causes of early decomposition of cream that results in a high WIA content.

Acidity of Cream. The activity of lipases produced by microorganisms and those normally present in milk and cream is decreased as the *p*H is lowered. Purko *et al.*[173] inoculated 3 lots of sterile cream, having a titratable acidity of 0.13% and a WIA value of 155, with *G. candidum*. The titratable acidities of 2 lots then was adjusted to 0.50 and 0.88%. After incubation for 3 days, the cream having acidities of 0.13, 0.50, and 0.88% contained 11,777, 8,381, and 2,064 mg WIA, respectively. In other investigations [174] pasteurized cream inoculated with *Ps. fluorescens* and held 5 days at 70 F yielded butter with a WIA content of 530, whereas cream containing *S. lactis* and a mixture of *S. lactis* and *Ps. fluorescens* gave butter with WIA values of 73 and 79, respectively. After incubation the samples containing *Ps. fluorescens*, *S. lactis*, and *S. lactis* plus *Ps. fluorescens* had titratable acidities of 0.19, 0.93, and 0.95%, respectively.

Neutralization of Cream. According to Hillig *et al.*,[185] neutralization of cream for butter making does not increase the WIA content. Four samples of butter had higher WIA contents than the cream from which they were churned, but the differences (35 to 49 mg) were not great. Feder and Breazeale [179] found no correlation between the *p*H and WIA content of butter within the *p*H range of 6.8 to 7.0. As the *p*H decreased from 6.8, the WIA values increased significantly. The lower *p*H values suggested that the cream was underneutralized and yielded butter with a high WIA content and fat acidity. The authors suggested careful neutralization of cream as a means of minimizing excessive WIA values. Parmelee and Babel [174] obtained lower WIA values on butter made from neutralized cream than from unneutralized; there was an indication that the more the acidity of the cream at churning is reduced, the more the WIA content of the resulting butter is reduced.

Method of Churning. Hillig and Ahlmann [186] determined the WIA content of butter churned from the same vat of cream by a continuous method and by a barrel churn; the methods gave butter with similar values. Freeman and Barkman [170] found that the "carry-over" of WIA from cream to finished butter ranged from 54 to 98%.

Relation of WIA to Fat Acidity. Since the method for determining WIA is time consuming, various investigators have compared fat acidities with WIA values. Results of colorimetric [187,188] and titration procedures [170,174,179,180,189] for fat acidity have shown a general relation to WIA values. A fat titration method for the rapid estimation of WIA in cream and butter was proposed by Hillig.[190] The method

requires about 15 min and WIA values are calculated from the titration value and average mean molecular weight of 270 for WIA.

Butyric Acid in Cream and Butter

The hydrolysis of fat in milk, cream, butter, or other dairy product commonly results in the liberation of some butyric acid since it is one of the acids making up the fat molecules. Under certain conditions butyric acid is synthesized by some micro-organisms. The butyric acid content of cream and butter also has been used by regulatory officials to determine decomposition in the products.

Butyric Acid in Cream. When Hillig applied a chromatographic procedure [191] for butyric acid to cream, he noted that the acid was present only in cream undergoing violent fermentation and having a disagreeable flavor. The butyric acid was believed to originate from lactose and not from fat hydrolysis. Butyric acid was not detected in cream suitable for butter, but it was present in poor-quality cream.[192] Peters [193] found butyric acid in fresh cream and the amount increased with incubation. Some butyric acid developed in cream held at 38 F, but considerably more developed at 85 F.

Butyric Acid in Butter. Commercial sweet-cream butter analyzed by Hillig and Montgomery [192] contained no determinable butyric acid. They concluded that the mere presence of butyric acid in butter indicated that some decomposed cream was present in the churning mixture. Butter churned from a mixture containing some poor-quality cream frequently contained butyric acid and had a high WIA value and mold mycelia count. Parmelee and Babel [174] found 0.09 to 0.26 mg butyric acid per 100 g fat in butter prepared from the fresh milk of individual cows. A statistical analysis of data obtained on 113 samples of butter showed a rather poor correlation between butyric acid and WIA.

*p*H Determinations on Butter Serum

*p*H determinations on butter serum, obtained by melting the butter at about 150 F and centrifuging, are most accurate when made potentiometrically, but satisfactory results also can be obtained colorimetrically, using bromthymol blue.[194]

Salted butter requires proper *p*H control for good keeping quality. With normal cream, adequate control of the titratable acidity of the cream at churning gives the desired *p*H in the butter serum, but unusual conditions may upset the relationship (see p. 475). In examin-

ing butter that shows an off flavor for the purpose of assigning the cause, a *p*H determination is advisable.

Phosphatase Tests on Butter

When the phosphatase test proved useful in controlling the pasteurization of milk, attempts were made to use it in the butter industry. However, some butter from well pasteurized cream was found phosphatase positive.

During separation of milk there is a concentration of phosphatase in the cream. In certain cases, separation of pasteurized, phosphatase-negative milk has given phosphatase-positive cream, due to concentration of residual phosphatase. When cream is churned there may be a concentration of residual phosphatase in the butter serum. Under these conditions a positive test on fresh butter may not indicate that the cream was heated insufficiently. Normally, cream for butter is pasteurized at a higher temperature than milk.

Much cream used for butter, even when sweet, has undergone considerable growth of organisms and may contain microbial phosphatase. Barber and Frazier [195] reported that bacterial phosphatase (*B. cereus* and *B. mesentericus*) and milk phosphatase were active in the same *p*H range, but in cream the former withstood exposures as high as 170 F for 30 min while the latter was inactivated at 145 F for 30 min. Thus a positive test on fresh butter could be due to microbial phosphatase.

Various bacteria sometimes grow rather rapidly in butter, especially fresh butter because of the temperature at which it comes from the churn and the slow cooling at the centers of the boxes, etc. Olson and Hammer [196] found that certain species produce bacterial phosphatase, or other products, in sufficient amounts to interfere with the testing of butter for milk phosphatase.

Because of the various irregularities, application of the phosphatase test to butter requires special considerations.

Keeping-quality Tests on Butter

A prime consideration with every lot of butter is the way it keeps. The failure of attempts to establish a correlation between numbers of various types of organisms in butter and its keeping quality has led to the suggestion [197] that small portions of butter be held under conditions favorable for spoilage and examined frequently for defects. In general, the defects this procedure is expected to detect are those due to organisms.

Various butter organizations have found keeping-quality tests useful in controlling their products. There is considerable variation in the procedures employed. Small portions of butter from churns or boxes can be placed in sterile containers but, since some lots of butter that keep well in an unprinted condition fail to keep after printing, the preferred method is to take wrapped prints from the print line so that the butter is in the condition for market. In some plants the samples are held at 70 F; others use a somewhat lower temperature. The butter is examined after various holding periods but usually the first examination is made after 2 or 3 days because of the advantage in detecting, as soon as possible, the lots of butter that will not keep. The final examination is made after 7 to 14 days. When subjected to keeping-quality tests many lots of butter become tallowy because of the effect of temperature on development of this defect, and ordinarily such a change is ignored in the tests.

Certain plants that market salted butter but do not print it have found it helpful to test the keeping quality of their product in an unsalted condition. Small portions are taken from the churns just before salting and held in sterile jars or parchment. The lack of salt compensates in part for the effect of printing on the keeping quality.

In some cases keeping-quality tests have been run on butter that has been melted so the serum is separated.[100] This introduces such a change in the physical state that spoilage may occur when it does not occur in unmelted butter. Jacobsen [198] noted that at 69.8 F, or room temperature, flavor defects appeared sooner in the serum of unsalted butter than in the corresponding butter and frequently were different in character.

Keeping-quality tests have been considered too sensitive by some users and not sensitive enough by others. The former group believes that certain lots of butter deteriorate rapidly at the temperatures used for the tests and keep satisfactorily under commercial conditions; the latter group believes that some lots of butter keep satisfactorily in the tests and still deteriorate under commercial conditions. Temperature has a definite effect on growth of organisms in butter, and a close correlation between deterioration at various temperatures cannot be expected. Extensive commercial experience shows that, with suitable pasteurization and minimum contamination of cream and butter, it is possible to manufacture butter that keeps satisfactorily, as far as biological defects are concerned, even under very adverse conditions and keeping-quality tests are helpful in detecting faulty methods of production. In employing the tests each sample of butter must be

examined critically; one defective churning among many that are satisfactory is missed easily. General experience indicates that before a serious outbreak of spoilage occurs in the product of a plant there frequently is a period during which an occasional churning shows poor keeping quality; if these are detected and corrective measures instituted, an outbreak may be avoided.

REFERENCES

1. *Standard Methods for the Examination of Dairy Products.* (Last ed.) American Public Health Association, Inc., New York, N. Y.
2. *Ia. Agr. Expt. Sta. Research Bull.* 137 (1931).
3. *B. W. Hammer Panegyric,* 73, 1937. Collegiate Press, Inc., Ames, Ia.
4. *Ia. State Coll. J. Sci.,* **12,** 441 (1938).
5. *Sci. Agr.,* **8,** 353 (1928).
6. *Can. Dept. Agr. Pamphlet* 92 (1928).
7. *J. Dairy Sci.,* **14,** 463 (1931).
8. *Irish Free State Dept. Agr. J.,* **28,** 199 (1929).
9. *J. Dairy Sci.,* **14,** 494 (1931).
10. *Natl. Butter J.,* **22,** 16 (1931).
11. *J. Dairy Sci.,* **14,** 398 (1931).
12. *Ia. Agr. Expt. Sta. Research Bull.* 159 (1933).
13. *J. Dairy Sci.,* **24,** 921 (1941).
14. *J. Dairy Sci.,* **17,** 613 (1934).
15. *Can. Dairy Ice Cream J.,* **22** (10), 25 (1943).
16. *J. Dairy Sci.,* **6,** 427 (1923).
17. *Minn. Agr. Expt. Sta. Tech. Bull.* 82 (1932).
18. Parfitt, E. H. A Study of Methods of Determining the Number of Yeasts and Molds in Butter. Thesis. Ia. State Coll. (1934).
19. *J. Dairy Sci.,* **27,** 563 (1944).
20. *J. Dairy Sci.,* **11,** 446 (1928).
21. *Ia. Agr. Expt. Sta. Research Bull.* 134 (1930).
22. *43rd Ann. Conf. Victoria Diary Factory Mgr. Sec. Assoc.,* 50 (1936).
23. *Ia. Agr. Expt. Sta. Research Bull.* 246 (1938).
24. *Australian J. Dairy Technol.,* **7,** 44 (1952).
25. *Ia. Agr. Expt. Sta. Research Bull.* 263 (1939).
26. *Ia. State Coll. J. Sci.,* **7,** 453 (1933).
27. *Intern. Dairy Congr., Proc. 12th Congr.,* **2,** 88 (1949).
28. *J. Dairy Sci.,* **16,** 375 (1933).
29. *Mich. Agr. Expt. Sta. Tech. Bull.* 2 (1909).
30. *Ia. Agr. Expt. Sta. Research Bull.* 339 (1945).
31. *Irish Free State Dept. Agr. J.,* **31,** 170 (1932).
32. *Irish Free State Dept. Agr. J.,* **32,** 257 (1933).
33. *J. Dairy Research,* **5,** 124 (1934).
34. *Address 31st Ann. Meeting Am. Butter Inst.* (1939).
35. *Irish Free State Dept. Agr. J.,* **31,** 226 (1932).
36. *J. Australian Inst. Agr. Sci.,* **4,** 96 (1938).
37. *Ia. Agr. Expt. Sta. Research Bull.* 169 (1934).
38. *Bacteriol. Revs.,* **5,** 337 (1941).
39. *J. Dairy Sci.,* **27,** 79 (1944).
40. *Biochem. Z.,* **210,** 234 (1929).
41. *Biochem. Z.,* **216,** 330 (1929).
42. *Off. Org. algem ned zuivelb.,* **14,** 110 (1949).
43. *Neth. Milk Dairy J.,* **2,** 25 (1948).

44. *J. Nutrition*, **27**, 339 (1944).
45. *J. Dairy Research*, **8**, 15 (1937).
46. *Proc. World's Dairy Congr., 11th Congr.*, **2**, 76 (1937).
47. *J. Dairy Sci.*, **22**, 79 (1939).
48. *Neth. Milk Dairy J.*, **6**, 195 (1952).
49. *Proc. World's Dairy Congr., 11th Congr.*, **2**, 85 (1937).
50. *Proc. World's Dairy Congr., 11th Congr.*, **2**, 121 (1937).
51. *J. Dairy Sci.*, **25**, 175 (1942).
52. *J. Dairy Sci.*, **23**, 159 (1940).
53. *U. S. Dept. Agr., Bur. Animal Ind., Bull.* 57 (1904).
54. *U. S. Dept. Agr., Bur. Animal Ind., Bull.* 162 (1913).
55. *J. Dairy Sci.*, **1**, 347 (1917).
56. *J. Agr. Research*, **11**, 437 (1917).
57. *U. S. Dept. Agr., Bur. Animal Ind., Bull.* 84 (1906).
58. *J. Agr. Research*, **6**, 927 (1916).
59. *New Zealand J. Sci. Technol.*, **29**, 193 (1948).
60. *Conn. (Storrs) Agr. Expt. Sta. 2nd Ann. Rept.*, 63 (1889).
61. *Ia. Agr. Expt. Sta. Bull.* 8 and 11 (1890).
62. *Ia. Agr. Expt. Sta. Bull.* 18 (1892).
63. *Ia. Agr. Expt. Sta. Bull.* 21 (1893).
64. *U. S. Dept. Agr. Circ.* 146 (1909).
65. *U. S. Dept. Agr., Bur. Animal Ind., Bull.* 114 (1909).
66. *U. S. Dept. Agr., Bur. Animal Ind., Bull.* 148 (1912).
67. *Ia. Agr. Expt. Sta. Bull.* 207 (1922).
68. *Dairy Produce*, **34**, 24 (Jan. 1928).
69. *Irish Free State Dept. Agr. J.*, **32** 273 (1933).
70. *Irish Free State Dept. Agr. J.*, **33**, 23 (1935).
71. *Sci. Agr.*, **25**, 137 (1944).
72. *J. Dairy Sci.*, **11**, 143 (1928).
73. *Landwirtsch. Jahrb.*, **51**, 489 (1918).
74. *J. Dairy Sci.*, **3**, 452 (1920).
75. *Australian Milk Dairy Products J.*, **14** (7), 39 (1947).
76. *J. Council Sci. Ind. Research*, **21**, 319 (1948).
77. *Can. Dairy Ice Cream J.*, **31** (4), 31 (1952).
78. *Lait*, **31**, 505 (1951).
79. *J. Dairy Sci.*, **7**, 484 (1924).
80. *J. Dairy Sci.*, **8**, 318 (1925).
81. *Ia. Agr. Expt. Sta. Research Bull.* 95 (1926).
82. *Neth. Milk Dairy J.*, **1**, 219 (1947).
83. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 373 (1926).
84. *Proc. Ia. Acad. Sci.*, **32**, 55 (1925).
85. *J. Dairy Sci.*, **20**, 463 (1937).
86. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **8**, 11, 74, 107, 140, 171, 211, 309, 342, 367, 406 (1902).
87. *Natl. Butter Cheese J.*, **33** (12), 16 (1942).
88. *J. Dairy Sci.*, **1**, 218 (1917).
89. *Agr. Gaz. N. S. Wales*, **12**, 341 (1901).
90. *Agr. Gaz. N. S. Wales*, **18**, 223 (1907).
91. *N. Y. Produce Rev.*, **13**, 20 (1901).
92. *J. New Zealand Dept. Agr.*, **4**, 1 (1912).
93. *N. Y. (Cornell) Agr. Expt. Sta. Mem.* 29 (1919).
94. *Wis. Agr. Expt. Sta. Research Bull.* 57 (1923).
95. *J. Dairy Sci.*, **3**, 194 (1920).
96. *Purdue Agr. Expt. Sta. Bull.* 401 (1935).
97. *J. Dairy Sci.*, **27**, 45 (1944).
98. *Ia. Agr. Expt. Sta. Bull.* 59 (1901).
99. *Can. Dept. Agr. Pamphlet* 91 (1928).
100. *Agr. Gaz. N. S. Wales*, **40**, 244 (1929).

101. *Ia. Agr. Expt. Sta. Research Bull.* 145 (1931).
102. *Ia. Agr. Expt. Sta. Research Bull.* 267 (1939).
103. *Sci. Agr.*, **21**, 133 (1940).
104. *Ia. Agr. Expt. Sta. Research Bull.* 285 (1941).
105. *J. Dairy Sci.*, **36**, 414 (1953).
106. *J. Dept. Agr. W. Australia*, **15**, 137 (1938).
107. *J. Council Sci. Ind. Research*, **14**, 1 (1941).
108. *J. Dept. Agr. Victoria*, **39**, 39 (1941).
109. *J. Dairy Research*, **17**, 66 (1950).
110. *J. Dairy Sci.*, **26**, 943 (1943).
111. *J. Dairy Sci.*, **28**, 93 (1945).
112. *Sci. Agr.*, **22**, 277 (1942).
113. *Sci. Agr.*, **22**, 347 (1942).
114. *Sci. Agr.*, **22**, 552 (1942).
115. *Sci. Agr.*, **22**, 637 (1942).
116. *Sci. Agr.*, **24**, 21 (1943)
117. *J. Dairy Sci.*, **1**, 320 (1917).
118. *J. Dairy Sci.*, **2**, 444 (1919).
119. *Food Research*, **9**, 289 (1944).
120. *Milchwirtsch. Forsch.*, **12**, 172 (1931).
121. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 382 (1949).
122. *J. Dairy Research*, **10**, 300 (1939).
123. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 472 (1949).
124. *J. Dairy Sci.*, **15**, 199 (1932).
125. *J. Bacteriol.*, **37**, 251 (1939).
126. *Okla. Agr. Expt. Sta. Bull.* B401 (1953).
127. *Ia. State Coll. J. Sci.*, **11**, 281 (1937).
128. *J. Dept. Agr. Victoria*, **39**, 283 (1941).
129. *J. Agr. Research*, **3**, 301 (1915).
130. *Minn. Agr. Expt. Sta. Bull.* 235 (1927).
131. *Can. Dept. Agr. Bull.* 48 (1925).
132. *J. Agr. Research*, **6**, 153 (1916).
133. *New Zealand J. Agr.*, **53**, 129 (1936).
134. *Milch-Ztg.*, **28**, 626, 644, 662 (1899).
135. *Address Wis. Buttermakers Assoc.*, Dec. 5, 1916.
136. *J. Dairy Sci.*, **23**, 509 (1940).
137. *J. Dairy Sci.*, **28**, 701 (1945).
138. *J. Dairy Sci.*, **29**, 173 (1946).
139. *Wis. Agr. Expt. Sta. Bull.* 461, 40 (1943).
140. *Food Inds.*, **17**, 622 (1945).
141. *J. Dairy Sci.*, **25**, 169 (1942).
142. *J. Invest. Dermatol.*, **1**, 237 (1938).
143. *Food Research*, **4**, 539 (1939).
144. *Food Technol.*, **4**, 294 (1950).
145. *Mich. Agr. Expt. Sta. Quart. Bull.*, **33**, 127 (1950).
146. *J. Dairy Research*, **7**, 238 (1936).
147. *Sci. Agr.*, **20**, 638 (1940).
148. *Can. Dairy Ice Cream J.*, **28** (7), 29 (1949).
149. *Ia. State Coll. J. Sci.*, **7**, 487 (1933).
150. *New Zealand J. Sci. Technol.*, **15**, 199 (1933).
151. *U. S. Public Health Mar. Hosp. Service Hyg. Lab. Bull.* 56, 739 (1909).
152. *J. Agr. Research*, **31**, 695 (1925).
153. *Ark. Agr. Expt. Sta. Bull.* 480 (1948).
154. *Ore. Agr. Expt. Sta. Bull.* 305 (1932).
155. *Kan. Agr. Expt. Sta. Circ.* 180 (1936).
156. *J. Assoc. Offic. Agr. Chemists*, **20**, 93 (1937).
157. *Publ. Am. Butter Inst.*, Chicago (1941).
158. *J. Dairy Sci.*, **22**, 367 (1939).
159. *Natl. Butter Cheese J.*, **32** (7), 8 (1941).
160. *J. Dairy Sci.*, **25**, 716 (1942).
161. *Ark. Agr. Expt. Sta. Bull.* 432 (1943).
162. *Purdue Agr. Expt. Sta. Bull.* 465 (1942).
163. *Mont. Agr. Expt. Sta. Bull.* 415 (1943).
164. *Natl. Butter Cheese J.*, **33** (6), 10 (1942).

165. *J. Dairy Sci.*, **24**, 548 (1941).
166. *J. Dairy Sci.*, **27**, 563 (1944).
167. *J. Dairy Sci.*, **28**, 519 (1945).
168. *J. Dairy Sci.*, **25**, 195 (1942).
169. *J. Assoc. Offic. Agr. Chemists*, **30**, 575 (1947).
170. *Am. Dairy Products Rev.*, **15**, 2 (1953).
171. *J. Dairy Sci.*, **33**, 398 (1950).
172. *J. Dairy Sci.*, **34**, 477 (1951).
173. *J. Dairy Sci.*, **35**, 298 (1952).
174. *Purdue Agr. Expt. Sta. Bull.* 603 (1953).
175. *J. Assoc. Offic. Agr. Chemists*, **35**, 844 (1952).
176. *J. Assoc. Offic. Agr. Chemists*, **33**, 716 (1950).
177. *J. Assoc. Offic. Agr. Chemists*, **32**, 731 (1949).
178. *Butter, Cheese, Milk Products J.*, **42** (5), 28 (1951).
179. *S. Dak. Farm Home Research*, **3** (2), 25 (1952).
180. *J. Dairy Sci.*, **34**, 478 (1951).
181. *J. Assoc. Offic. Agr. Chemists*, **31**, 739 (1948).
182. *J. Dairy Sci.*, **36**, 794 (1953).
183. *J. Assoc. Offic. Agr. Chemists*, **34**, 782 (1951).
184. *J. Assoc. Offic. Agr. Chemists*, **35**, 852 (1952).
185. *J. Assoc. Offic. Agr. Chemists*, **34**, 720 (1951).
186. *J. Assoc. Offic. Agr. Chemists*, **34**, 777 (1951).
187. *J. Dairy Sci.*, **35**, 59 (1952).
188. *J. Dairy Sci.*, **34**, 477 (1951).
189. *Milk Products J.*, **45** (5), 37 (1954).
190. *J. Assoc. Offic. Agr. Chemists*, **36**, 1077 (1953).
191. *J. Assoc. Offic. Agr. Chemists*, **31**, 750 (1948).
192. *J. Assoc. Offic. Agr. Chemists*, **31**, 755 (1948).
193. *J. Dairy Sci.*, **36**, 268 (1953).
194. *Ia. Agr. Expt. Sta. Research Bull.* 316 (1943).
195. *J. Dairy Sci.*, **26**, 343 (1943).
196. *J. Milk Technol.*, **4**, 83 (1941).
197. *Univ. Wis. Studies Sci.*, No. 2, 131 (1921).
198. *S. Dak. Agr. Expt. Sta. Bull.* 308 (1937).

Bacteriology of Cheeses

19

The possibility of preparing cheese from milk apparently was discovered by accident when milk was held in a container made of the stomach of a calf, where it was coagulated through the action of rennin. More or less definite types of cheeses originated through variations in temperatures used in manufacture, in water, fat, and salt contents, and in ripening conditions since all these factors have a pronounced effect on the species of organisms developing in the product and, accordingly, on the chemical changes induced.* Although methods of making cheeses were developed long before the importance of organisms in causing changes in dairy products was recognized, study of the action of various species in cheese ripening has resulted in improvements in the manufacturing procedures and has made it possible to produce successfully various cheeses in localities that lack some of the natural advantages under which the making began.

The changes involved in the ripening of certain cheeses constitute the most complex microbiological processes in the field of dairying. Action of large numbers of organisms of various species is required and

* With each variety of cheese the method of making often varies somewhat from plant to plant. Brief descriptions of the processes used for many cheeses are given in "Cheese Varieties," U. S. Dept. Agr., *Agr. Handbook, No.* 54, 1953.

often it is difficult to evaluate the exact role of the different species. Study of the ripening processes is complicated by the presence in cheeses of organisms to which it is difficult to assign a definite significance but which may be of importance. The numerous unsolved problems in connection with cheese ripening indicate the difficulties presented.

Classification of Cheeses. Many varieties of cheeses are recognized [1] but often the differences between two or even several varieties are insignificant and there are only relatively few basic types. With most varieties, rennin in the form of commercial rennet extract is used to coagulate the milk, and a portion of the water and materials in solution in it are drained away as whey. Cheeses can be divided into hard, semihard, and soft groups, depending largely on the moisture contents of the finished products. Hard cheeses, such as cheddar and swiss, are primarily bacteria ripened; of the semihard cheeses, brick is primarily bacteria ripened, and blue, roquefort, and gorgonzola are primarily mold ripened; and of the soft cheeses, limburger is primarily bacteria ripened, camembert is primarily mold ripened, and cottage and cream are unripened. In addition to the natural cheeses, processed cheeses, cheese spreads, and cheese foods are used in large amounts.

With the typical hard cheeses ripening goes on quite uniformly throughout the mass, except as influenced by such factors as drying at the surface, and if desired they can be made in large sizes. The properly made and ripened products are not susceptible to rapid action of harmful organisms and have good keeping quality. With the ripened soft cheeses micro-organisms prominently connected with the ripening develop at the surface and the products of growth diffuse through the cheese mass and effect the characteristic changes; thus, the necessary diffusion limits the sizes of the cheeses. With these cheeses the opportunity for continued growth of micro-organisms, even after the desired ripening has occurred, results in development of various defects and the keeping quality is limited. In the unripened soft cheeses, flavor development largely occurs during manufacture. Although there may be an increase in the pleasing flavor for a period after the making, extensive growth of organisms commonly results in loss of the desired flavor and in other objectionable changes. The semihard cheeses require definite ripening through the action of organisms and, in general, they rank between the hard and the soft groups in keeping quality.

General Ripening Agents. The general changes that occur in cheeses during ripening involve action of micro-organisms and of enzymes normal to milk or added with the coagulating agent. More than one species of organism ordinarily is concerned with the ripening of a variety and the changes brought about by the various species may be quite different. Products resulting from the action of one ripening agent sometimes make possible a later change, and in this way a regular sequence of changes may occur.

Some of the early work on cheese ripening led to erroneous conclusions as to the responsible agents. Bits of old cheese were inoculated into sterile milk and caused coagulation and a cheesy odor which led to the assumption that aerobic, spore-bearing rods developing in the sterile milk were an important factor in the ripening. These organisms controlled the fermentation in the inoculated milk because the bacteria that are important in the ripening do not survive the conditions existing in old cheese in such a way that they can grow rapidly when introduced into milk. Later investigations showed the presence of large numbers of acid-forming bacteria during ripening of various cheeses and indicated the importance of such types. In general, the organisms that grow well in milk also grow well in cheese; normally, the first step in the making and ripening of various cheeses is development of acid through action of bacteria, usually one of the streptococci.

To insure an abundance of acid-producing streptococci in cheese, a lactic culture commonly is added to the milk. Coagulation of milk with rennet or acid concentrates these organisms, as well as others, in the curd, and the whey commonly contains smaller numbers per milliliter than the milk. In laboratory trials Hastings *et al.*[2] found that 3 lots of milk contained 128, 11, and 2.05 million bacteria per ml, whereas the corresponding whey contained 34, 2.9, and 0.73 million. In the laboratory trials about 77% of the organisms were held in the curd and under practical conditions about 73%.

Because organisms are concentrated in the curd, the production of acid there is relatively rapid. In trials by Hastings *et al.*,[2] deep beakers were filled with milk, the milk curdled with rennet, the curd cut and allowed to settle, and then the acidity of the whey at the top and bottom determined after various periods. Acidities at the bottom of a beaker regularly were higher than at the top which indicates that acid production was rapid in the curd. This activity of the lactic acid organisms in the curd undoubtedly persists during the making and early ripening of various cheeses.

Distribution of Organisms in Cheeses. When milk is coagulated, with either rennet or acid, the organisms in the curd are held firmly, so that the growth which occurs later results in formation of colonies comparable to the colonies in agar. Accordingly, the organisms in a cheese are more or less unevenly distributed and, whereas a species that was very numerous in the milk is present in each tiny portion of the cheese, another type may not be present in a relatively large portion, even after extensive multiplication. Growth of an important organism may be limited to a certain part of the cheese by some such factor as air supply; this is illustrated best by the cheeses in which development of molds normally occurs. There is diffusion of materials to and from the colonies of organisms so that ripening is not confined to the immediate neighborhood of the masses of growth.

Determination of Number of Bacteria in Cheese. The number of bacteria per gram of cheese ordinarily is determined by the plate method. The portion for examination, obtained with precautions against contamination, is weighed on a sterile paper and ground in a sterile mortar with some material which helps to disintegrate it, after which the mass is transferred to a dilution blank. The blank is thoroughly shaken and used as with milk. Approximately 1 g is a convenient sample, but the exact quantity is immaterial if the weight is known so that the final result can be calculated to a 1-g basis. Ordinarily, the sample can be removed rather completely from the paper; if not, the paper is ground with the cheese and then it is protected by having a larger sterile paper on the balance pan.

A 3-in. mortar is a satisfactory size for grinding cheese. The mortar and pestle can be wrapped together in paper and sterilized. Fine sea sand has been used extensively as a grinding agent and can be sterilized in cotton-stoppered test tubes. Sodium citrate solution frequently is preferred. A 2% aqueous solution can be sterilized and the desired quantity employed directly for disintegrating the cheese in the mortar. By adding the grinding material to the mortar in small amounts, the cheese can be broken up more easily than when it is added in one lot. The cheese must be thoroughly divided and this requires extensive grinding. If the dilution blank contains 1% sodium citrate and a quantity of glass beads, disintegration of the cheese ordinarily is more complete on thorough shaking than when water is used alone. Appearance of the material in the dilution blank shows how completely the cheese has been disintegrated.

In examining soft cheeses, the surface should be avoided because of the abundance of organisms there, unless it is of special interest when

it best is examined separately. Blue-veined cheeses, in which there normally is an uneven distribution of the characteristic organism, are especially difficult to sample for microbiological examination.

Any medium giving adequate growth on plates poured with milk is satisfactory for general counts on cheese, but special media may be needed in examinations for certain species, such as propionic acid organisms or yeasts and molds. In studying the presence of organisms that are not aerobic, shake cultures, or plates with limited air supply, are necessary. With most cheeses, better colony development is obtained at room temperature, 69.8 F (21 C), or 86 F (30 C) for 3 to 5 days than at 98.6 F (37 C).

Because of the great difficulty in completely breaking up the colonies existing in a mass of cheese, plate counts on cheese regularly give results that are low. However, data obtained under controlled conditions on different lots of cheese, or on the same lot at different periods, show general changes in both the numbers of organisms and the important types. They also often help detect species responsible for defects in cheese.

For rough determinations of the numbers of certain organisms, such as *L. casei*, that develop poorly on plates but well in milk, addition of dilutions of ground cheese to tubes of sterile milk has been employed. The tubes are held at a temperature favorable for growth and then examined in the gross and also microscopically in order to obtain information on the morphologic types in the various dilutions. Approximations of the numbers of organisms of the different types then can be made.

Valuable data on the organisms in cheese sometimes are obtained by making direct microscopic examinations. A piece of cheese with a smooth surface can be pressed firmly against a clean slide and then removed so as to leave organisms distributed on the slide in much the way they were on the surface of the cheese. The "impression" then is fixed, freed of fat, stained, and examined. For the purpose of showing that important morphologic types are not being missed by the plate method, a microscopic examination is as valuable with cheese as with other materials. Actual microscopic counts of the organisms in cheese can be obtained by making smears of the cheese dispersed in dilution blanks containing sodium citrate and beads, removing the fat, staining, and counting.

Certain investigators have cut sections of cheese for microscopic examination in the way that sections of tissue are cut. Hucker [3] used such a procedure with cheddar cheese and noted that the numbers of

organisms determined by this direct method were much higher than plate counts.

Quality of Milk for Cheese. Although the milk used for cheese often is produced and handled under less rigid conditions than that for direct consumption, the quality still is an important consideration. When the milk contains large numbers of organisms or shows other defects, the cheese often is of inferior quality both as to flavor and body and texture, even when pasteurization has been used. Fine cheese requires high-quality milk as well as proper making and handling.

Certain poor-quality feeds may be a factor in producing milk for cheese. For example, grass silage that is low in acid may contain gas-producing organisms which harm cheese quality if they get into the milk.

Cheese Plant Sanitation. In a cheese plant, sanitation is of extreme importance since objectionable organisms added to the milk or curd may grow during the making and holding of the cheese. Pipe lines, pumps, and vats, and such equipment as rakes, forks, strainers, scoops, etc., require proper cleaning and sanitizing. Small equipment can be sanitized just before use or, preferably, placed in a steam chamber at the end of the day, heated thoroughly, and left until needed. The pasteurization should be checked frequently to avoid irregularities in the heating. Since employees work over the milk and curd so much, they should be especially concerned with the cleanliness of their hands, arms, and clothing and should use head coverings. Curd mills, agitators, etc., need constant attention if they are to be kept in a satisfactory condition. Hoops must be kept clean to avoid contamination of the curd and to simplify removal of the cheese from them. The need for attention to details in plant operation is evident from the frequency with which microbiological defects occur in cheese.

CHEDDAR CHEESE

Cheddar cheese is a hard variety, originally made in England, in which normal ripening involves action of various bacteria, without formation of eyes. Each of the necessary types brings about a change that is important in the ripening process. Other species of organisms commonly are present; certain of them are unimportant, whereas others may cause defects.

Raw-milk Cheese. Much excellent cheese has been made from raw milk of satisfactory quality. As the area producing milk for a plant

expanded and as cheese production moved into the warmer sections of the United States, more difficulty was encountered in getting raw milk to the vats in good condition, and cheese defects, especially gassiness, became more frequent. This situation, and more recently the spread of such diseases as typhoid fever through young raw-milk cheese, suggested that milk for cheese should be pasteurized. Various regulations now require that either the milk for cheese be pasteurized or the cheese be aged for a certain period which it is assumed will destroy any common disease-producing bacteria present.

Pasteurized-milk Cheese. Pasteurization first was employed extensively with cheese milk in New Zealand, and the cheese made proved satisfactory on the important markets of the world. Sawers,[4] in 1925, pointed out that pasteurization of milk for cheese in New Zealand resulted in a product of higher quality, which maintained its quality better under storage, and which also stood transit better. Pasteurization of milk for cheese soon spread to the United States and other countries.

Walter and Lochry,[5] in 1945, noted that extensive use of pasteurization in the manufacture of cheddar cheese had demonstrated that a high percentage of No. 1 cheese can be made from pasteurized milk with a suitably controlled amount of active culture and a definite time schedule. Time schedules now are used widely in making pasteurized-milk cheese and, with uniform culture from day to day, help simplify and standardize the process. Attempts constantly are being made to shorten schedules so that labor costs can be reduced and vats freed for another lot of milk.

Establishing Acid Production. Lactic culture is added to the cheese milk and permitted to grow for a short period before coagulating the milk with rennet. This tends to ensure adequate acid production during manufacture and early holding of the cheese.

Importance of Acid Development. *S. lactis* is the first species to bring about an important change during the making and ripening of cheese. Production of acid begins early in the making process and normally continues as long as lactose is available. Because of the relatively high buffer capacity of cheese in relation to the lactose content, the *p*H change ordinarily does not prevent growth of *S. lactis* with lactose still present. Lactic acid is important in the manufacture and ripening of cheese because of the following effects: [2]

1. Favors Curdling of the Milk with Rennet. More rapid coagulation of milk with rennet is obtained when there is some developed acid present.

2. Favors Expulsion of the Whey. Proper expulsion of the whey is necessary to reduce the moisture content of the cheese to a satisfactory level. If the moisture content remains too high, the cheese commonly has an off flavor and fails to develop the desired character. Rapid production of acid aids materially in expelling whey.

3. Favors Fusion of the Curd Particles. Fusion of the curd particles is necessary if the cheese is to have the proper body. The particles must lose their identity and form a compact mass, rather than show a tendency to retain their forms. Proper fusion is favored by acid and, without it, the cheese often is crumbly.

4. Exerts a Protective Action against Putrefaction. Although putrefactive bacteria of various types often are present in cheese, they normally are prevented from growing, largely because of the acid present. Babcock *et al.*[6] noted that when curd was washed free of acid, and of lactose from which acid could be formed, it decomposed rather quickly.

5. Favors Proteolytic Action of Rennet Extract. Rennet extract aids in the protein breakdown in cheese, and this action is favored by acid. The general effect can be observed by adding 2 ml of sterile milk to each of 2 tubes of melted agar, inoculating one with a small amount of *S. lactis* culture, and then emptying each tube into a sterile petri dish. After incubating, a narrow strip of filter paper moistened with rennet extract is placed on each agar surface; both dishes then are held at 98.6 F. In a short period the dish in which *S. lactis* has produced acid will show a clear area around the paper, whereas the dish in which there has been no acid formation will show little if any clearing.

If a distinction is made between *S. lactis* and *S. cremoris* it apparently makes little difference which organism is employed in cheese making. Kelly[7] concluded that acid production appears to be the important function of the culture used in cheese and that satisfactory cheese can be made by inoculating the milk with either *S. lactis* or *S. cremoris*.

Changes in Numbers of Bacteria During Early Ripening. After the curd goes to the press, the culture organisms and often other species continue to grow actively. Plate counts obtained on young cheese undoubtedly are much too low, but they indicate the general trend in changes in numbers of bacteria. Harrison and Connell,[8] employing beef peptone gelatin or peptone whey gelatin, usually found the highest bacterial count on normal cheese when it was 1 day old, although

occasionally the maximum occurred in 2 to 5 days; the highest number of bacteria noted was 635,000,000 per g. Lactic acid organisms regularly predominated. Harding and Prucha [9] obtained similar data. *S. lactis* was the only species always found and it made up more than 99% of the total organisms. Hastings *et al.*[2] also noted that the maximum number of bacteria, as measured by lactose agar plates, occurred early in the ripening; in 8 of 11 cheese examined, the largest number of bacteria was found within 48 hr of making. Some counts were over 1,000,000,000 per g, and the maximum was 2,518,000,000. More recent data likewise show that the highest plate counts are obtained on cheese shortly after it is made and are followed by decreases. These are rapid at first but much slower later; they involve principally *S. lactis.*

Because of the influence of various factors, there undoubtedly is considerable variation in the maximum number of organisms in different lots of cheese and in the time this maximum is reached. Lactose greatly favors growth of *S. lactis,* and the amount of it retained is influenced by the moisture content of the cheese. It is probable that during early ripening the maximum number of organisms often is reached about when the lactose disappears. Temperature of holding would be expected to influence the rate of growth of organisms. Dolby *et al.*[10] found that the quantity of lactose incorporated in cheese is of importance in determining the acidity. When the lactose content was increased by adding it either to the milk or the curd, there was a more acid type of cheese at maturity although some lactose still was present in the cheese after 4 months.

Lactobacilli. The lactobacilli also are important in cheese and may reach numbers of 1 to 2 billion, or more, per g. They grow so poorly on certain media, particularly when growth must start from a single cell or small clump instead of from a mass of cells, that they probably have been missed or underestimated in various investigations. Studies on the comparative numbers of *S. lactis* and lactobacilli in cheese at different ages [2] indicate that *S. lactis* is important early in the ripening whereas the lactobacilli are dominant later, when the numbers of *S. lactis* have greatly decreased.

The lactobacilli in raw-milk cheese may differ from those in pasteurized-milk cheese. Slatter and Halvorson [11] found the majority of the former were killed at 143 F for 30 min or at 160 F for 15 sec, whereas the latter had a death time at 154 F of 10 to 35 min.

Conditions Favoring Growth of Lactobacilli. Growth of lactobacilli in cheese occurs, for the most part, after the lactose has been used by *S. lactis;* presumably, in cheese as in milk *S. lactis* is capable of more

rapid development than the lactobacilli, so that growth of lactobacilli is limited largely to a period when conditions have become unsatisfactory for *S. lactis*. Addition of peptone to milk favors acid production by various lactobacilli. Since the changes occurring in cheese ripening result in formation of protein decomposition products, including peptone, development of lactobacilli at a period when lactose no longer is available may be accelerated by accumulation of protein decomposition products.

Another factor of importance may be associative action of organisms. Hansen [12] noted that stimulation of *L. casei* by *S. lactis* or *S. cremoris* may play a role in the ripening of cheeses in which the two latter species develop, die, disintegrate, and are followed by *L. casei*.

Changes Produced by Lactobacilli. An important change effected in cheese by the lactobacilli is in connection with the breakdown of protein. Growth of the organisms in milk results in an increase in the soluble nitrogen that often is considerable, percentage-wise, especially with extended incubation, and the same general action probably occurs in cheese.

Allen and Knowles [13] concluded tentatively that one of the factors essential in inducing proper ripening in cheese made from milk of low bacterial count is lactobacilli of a type that will extend the protein decomposition initiated by enzymes and streptococci. Lane and Hammer [14] found with 6 of 8 strains of *L. casei* added individually to pasteurized milk made into cheese that there was a more rapid and extensive decomposition of the protein than occurred in cheese made without added lactobacilli; 2 strains had little effect on the decomposition. Flavor scores on the cheese made with *L. casei* commonly were more uniform and higher than those on the control cheese. Three of the strains of *L. casei* gave a typical cheddar cheese flavor while 3 others gave a pleasing, butter-like flavor suggestive of biacetyl but not typical of cheddar cheese. Phillips [15] noted that, in cheese having a *p*H value as low as 5.0 at 4 days, there was an improvement in flavor when the lactic culture was supplemented with lactobacilli.

Sherwood [16] found that, when inoculated into milk for cheese, different strains of lactobacilli imparted specifically characteristic flavors to the cheese; these included both desirable and undesirable flavors, many of which commonly are encountered in commercial cheese, such as pleasant aromatic, biacetyl-like, metallic, bitter, unclean, and fermented. Later, it was reported [17] that a significant feature of the ripening of New Zealand cheese is the early displacement of the lactic acid streptococci by lactobacilli. When clean milk (plate count after

pasteurization <2,000 per ml) is employed, lactobacilli usually make their appearance approximately 2 weeks after manufacture, but in commercial cheese the organisms dominate the flora earlier. It also was noted [18] that improvement of cheese by inoculation of good-quality milk with selected strains of lactobacilli gave the best results with relatively small inoculations.

According to McDonald,[19] development of mature flavor in cheese can be hastened by adding cultures of *L. casei* during manufacture. The increased rate of flavor development was most marked when whole milk cultures were added to the cheese in the later stages of manufacture, but the flavors that developed as a result of this technic were not uniform and off flavors may occur. The best-controlled improvement in rate of development of mature flavor took place when whey was used as the basic medium (various additions being made) for growth of cultures of lactobacilli, but no means could be devised to make the whey-base medium a practical procedure.

Yamamoto *et al.*[20] significantly improved the flavor of cheese by adding 0.05% of a strain of *L. bulgaricus* to *S. lactis* when inoculating the milk. More of the *L. bulgaricus* culture was less favorable. A strain of *L. casei* isolated from fine cheese had an adverse effect when added to milk for cheese.

Use of Lactobacilli in Cheese. The difficulties in classifying the lactobacilli isolated from cheese and comparable materials complicate the study of their value in cheese ripening. Moreover, two strains that belong to the same species may have different effects on the flavor of cheese; this situation obtains with other organisms producing flavor in dairy products. The large numbers of lactobacilli in many lots of fine raw-milk cheese suggest that these organisms are important in the ripening, and the trials in which cultures of lactobacilli have been used in cheese milk indicate their value if selected strains are used under suitable conditions.

Additional Organisms. In addition to *S. lactis* and lactobacilli, various groups of organisms are found more or less frequently in cheese. Some species in each group may play a part in the ripening, others probably are inert, and still others may cause defects. It is probable that different organisms are involved in the production of the desirable flavor in different lots of cheese, all with a fine flavor. There even may be some variation in the flavor constituents produced, with the various types equally satisfactory.

Streptococci other than *S. lactis* and micrococci are common in cheese. In milk some species in each group form products that also

are formed in cheese ripening which suggests their possible importance.

Protein Decomposition. From the chemical standpoint one of the important changes in the ripening of cheese is the breakdown of the casein derivatives. The tough, green curd must undergo protein degradation before it becomes a product that is recognized as ripened cheese. The breakdown is effected by enzymes and bacteria; the enzymes are protease and those in rennet extract, and various bacteria are involved.

Enzymes. Protease increases the soluble nitrogen in milk held with a reagent that prevents bacterial growth, and this suggests that protease is important in the protein decomposition during cheese ripening. Many of the investigations of this enzyme have been stimulated by the cheese-ripening problem, but the exact influence it has in cheese is difficult to determine because of the activity of other proteolytic agents. With protease, protein decomposition goes to the amino acid stage and considerable nitrogen in this form is one of the characteristics of certain hard cheeses.

During the early studies on cheese ripening there was a difference of opinion as to whether rennet extract has an effect in addition to coagulating the milk. Trials [21] indicated that an increase in the amount of rennet used caused an increase in the soluble nitrogenous products formed. Results of Babcock *et al.*[22] show this same effect and also that the increase in the products formed is confined to the higher decomposition bodies peculiar to pepsin, thus suggesting that the digestive action of rennet extract is attributable to pepsin in it. In cheese made from milk to which purified pepsin had been added, either with or without use of rennet to curdle the milk, the protein decomposition was increased.

Although rennet extract often is assumed to contain pepsin, certain results suggest it does not. However, Alais *et al.*[23] added the extract and also crystalline rennin (a highly purified product) to skim milk and, with both preparations, there was a rapid increase in nonprotein nitrogen.

Riddet *et al.*[24] found that an increased amount of rennet gave a more rapid breakdown in the earlier stages of cheese ripening and an increase in the proportion of soluble nitrogen, but there was a tendency to accentuate off flavors and to develop a sticky body in the mature cheese. The trials indicated that a 30% increase over the normal quantity of rennet would be an advantage when the cheese is to be sold for consumption soon after manufacture. Other investiga-

tions [25,26] also show that a more rapid breakdown of cheese can be obtained with an increased amount of rennet. However, the flavor developed may differ somewhat from that occurring with the normal amount of rennet and longer ripening. Babel [27] noted that the use of calcium chloride in cheese manufacture, for the purpose of decreasing the amount of rennet needed for coagulation, results in slower ripening which can be detected by organoleptic examination and by soluble nitrogen determinations.

Bacteria. Van Slyke *et al.*[28] made cheese from milk to which 4 to 5% chloroform had been added and then held the cheese in an atmosphere of chloroform. The soluble nitrogen formed was less than in normal cheese and the proportion of albumoses and peptones was high in comparison with the amides, whereas in normal cheese the amides were high. The results show the effect of enzymes on the ripening process and also indicate that bacterial action is necessary for the normal changes.

Among the organisms involved in protein decomposition in cheese, the lactobacilli are especially important. *S. lactis* also has the ability to increase the soluble nitrogen in milk and, presumably, does the same in cheese.

With 6 commercial cheese Kelly [29] found a steady breakdown of the proteins. In the ripened cheese the nonprotein nitrogen was never more than 24% of the total nitrogen. The increase in protein digestion after about 10 days seemed to be related to the destruction of bacteria; it may be that proteolytic enzymes are released from bacteria and aid in the protein hydrolysis. Davis *et al.*[30] reported that, in milk, the enzymes of commercial rennet, in conjunction with the lactic acid bacteria, can bring about protein breakdown similar in extent to that found in ripened cheese, as far as the nonprotein nitrogen is concerned. The amino nitrogen produced is much less than in cheese which may be due to the higher *p*H in the cheese.

Baribo and Foster [31] compared the characteristics of the intracellular proteinases of one strain each of *L. casei*, *S. lactis*, and *M. freudenreichii* with those of the proteolytic enzymes extracted from a year-old cheddar cheese and concluded that the organisms possessed enzymes which could account for only a part of the proteinases found in the cheese. It was suggested that rennet may provide part or all of the other proteolytic enzymes.

The general character of the protein decomposition in the ripening of cheddar cheese is shown by the early data of Van Slyke and Hart; [32] even with extended ripening, less than half the nitrogen was in water-

soluble forms, and amides made up a considerable portion of the water-soluble nitrogen. This type of protein decomposition would be favored by action of protease and certain bacteria.

Action of Salt. The salt added to the curd affects the body and texture and also the ripening of cheese. Riddet *et al.*[33] noted that, with undersalting, cheese had a pasty and weak body, open texture, and did not ripen normally; with oversalting, the cheese had a harsh body, ripened slowly, and the rind often cracked badly. McDowall and Whelan [34] reported that, in milk, growth of streptococci was inhibited in progressively greater degrees by salt concentrations from 3 to 6%. Five per cent gave definite but incomplete inhibition; this concentration corresponds to the limit of salt concentration in cheese of good body, which suggests that the effect of high salt on the body of cheese may be due to retarding the growth of lactic acid streptococci.

Immediately after salting, the surfaces and centers of pieces of curd have very different salt contents. With a microprocedure Hoecker and Hammer [35] found that at hooping the salt contents were relatively high at the surfaces of pieces of curd and comparatively low at the centers. During early pressing, rapid decreases in salt contents occurred at the surfaces and rapid increases at the centers; 24 hr after removal of the cheese from the press, differences in salt contents between surfaces and centers of pieces of curd usually were small. The *p*H values at milling were essentially the same at the surfaces and centers of pieces of curd. For a time after hooping, *p*H values were lower at the centers than at the surfaces but the differences rapidly became less, and 24 hr after removal of the cheese from the press they were small. The general results suggest that the effect of salt on the growth of organisms is relatively uniform throughout a cheese.

Importance of *p*H. The *p*H of cheese is of great importance from the standpoint of quality of the product. In general, the *p*H increases during extended ripening. Brown and Price [36] found a slight decrease in *p*H of cheese for some days after making and then a slow increase that became very definite with long ripening. They also noted that the quality of cheese is closely related to the acid development during the making; when the quality is inferior, the *p*H is low and the titratable acidity high at each critical point. Other investigators [37] have reported the effect of low *p*H in decreasing cheese quality and have emphasized [38] the importance of *p*H control in manufacture.

Chemical Compounds in Cheese. Various chemical compounds have been found in cheese, some of which contribute to the desirable flavor.

The amount of lactic acid in cheese has been reported [39] to fluctuate during the ripening; it usually was the racemic form. The acid that develops long after the lactose has disappeared may be of protein origin. Presumably, a decrease in the lactic acid in cheese is due to action of organisms.

There appears to be a rise and then a fall in the volatile acidity during the ripening of cheese. Suzuki *et al.*[39] found that acetic and propionic acids reached a maximum at 3 months and then decreased, whereas butyric and caproic acids increased continuously during the period of study. Formic acid was found only once. Lactates probably were the principal source of acetic and propionic acids, whereas fats and proteins were the sources of butyric and caproic acids. Ground cheese was distilled with steam after moistening it with dilute sulfuric acid and redistilled following neutralization with barium hydroxide. The final distillate was characterized by an odor resembling that of cheese and it contained alcohols and esters.

Succinic acid was isolated from ripening cheese and identified by its silver salt.[39] It also was obtained from a solution of lactose and peptone, fermented in the presence of calcium carbonate and fat, by inoculation of a bit of cheese.

The presence in cheese of specific amino acids has been shown by Tuckey *et al.*,[40] who found that, by extraction of aged cheese in hydrated *n*-butyl alcohol, amino acids were extracted and could be identified with x-ray analysis. Leucine and isoleucine were obtained from cheese 24 hr after being made. Tyrosine and probably tryptophane were identified in the extract obtained from ripened cheese. Baker and Nelson [41] reported that addition of various amino acids to cheddar cheese curd, made from pasteurized milk, shortly before it was placed in hoops had no consistent desirable effects on flavor. Kosikowsky [42] found the appearance of many free amino acids was very rapid in both raw- and pasteurized-milk cheese. At the end of 2 days at 60 F, 40% of the number of free amino acids that eventually were found in the cheese had appeared.

Tyramine was found in each of 25 varied samples of cheddar cheese by Kosikowsky and Dahlberg,[43] the average being 0.0384%, the minimum 0.0025, and the maximum 0.1199. It also was noted in miscellaneous varieties of ripened cheeses. Tyramine was not considered the flavor compound of cheddar cheese, but it served as a measure of bacterial activity that accentuated flavor production.[44]

Calbert and Price [45] found biacetyl in all lots and kinds of cheese examined and considered that a small amount contributes to the flavor

of cheddar cheese. Most lots of cheddar cheese with an excellent flavor had <0.05 mg biacetyl per 100 g. Larger amounts frequently appeared to be associated with flavor defects. Biacetyl was produced [46] throughout the manufacturing process, except between setting and cutting. It probably comes from the action of organisms on citrates. A gradual decrease in biacetyl was noted with both raw- and pasteurized-milk cheese during early curing and may have been due to volatilization and reduction.

White Particles in Cheese. Old cheese occasionally shows white particles. These have been reported as principally tyrosine,[47] which previously was noted in roquefort cheese, and also as calcium lactate.[48,49] Harper *et al.*[50] recently found that the particles are definitely heterogeneous in composition. The compounds found most commonly were tyrosine and calcium lactate. Cystine was found in the particles from 3 of 6 samples of cheese. Those from 1 sample contained leucine and probably isoleucine.

Defects of Cheddar Cheese

The conditions permitting extended growth of the desirable organisms during the making and ripening of cheese also permit growth of certain undesirable species when they are present, and this may result in cheese defects. Pasteurization of the milk tends to control objectionable organisms but, due to inadequate heating, recontamination, etc., has not controlled them completely.

Lack of Acid Development. Due to various causes (see p. 587), acid development sometimes is slow during the making of cheese. As a result, growth of certain objectionable bacteria is not restrained as it would be with normal acid formation. Also, large amounts of moisture and materials in solution, especially lactose, may be retained and, eventually, the cheese may have a high acid flavor, poor texture, etc.

Gassy Cheese. Development of gas holes is a common defect of cheese. *Escherichia-Aerobacter* organisms are most often responsible, but lactose-fermenting yeasts have been involved in some cases and spore-forming anaerobes in others.

Normally, some gas is produced in a cheese but this develops so slowly that it diffuses from the cheese about as fast as formed and gas holes do not result. Van Slyke and Hart [51] found that during ripening of a normal cheese carbon dioxide was given off continuously, although in decreasing amounts after about 20 weeks. The total amount produced was 15.1 g, equivalent to 0.5% of the fresh cheese. In a cheese

made from milk containing chloroform and kept under antiseptic conditions, the total amount of carbon dioxide produced was 0.25 g, practically none of which was formed after 3 weeks. It appeared that with the normal cheese the carbon dioxide given off early in the ripening came from decomposition of lactose by lactic acid organisms, although a small amount probably was attributable to gas present in the milk and to respiration of organisms in the cheese; the carbon dioxide produced later apparently came from certain chemical reactions.

Figure 44. Cheddar cheese showing gas formation.

Dorn and Dahlberg [52] found that, in tins, cheese from pasteurized high-quality milk produced less gas than cheese from raw milk and also was more consistent from batch to batch. Gas evolution was more rapid at 50 F than at 40 F; it was most rapid for approximately the first 4 weeks but never ceased even after 48 weeks. The gas almost exclusively was carbon dioxide. At 60 F, cheese removed nearly all the oxygen from a can in 1 week, but at 40 F about 7 weeks were required. Evolution of carbon dioxide appeared to bear an inverse relationship to loss of oxygen. Loss in weight of cheese due to carbon dioxide evolution varied from 0.13 to 0.20% for raw-milk cheese and from 0.04 to 0.09% for pasteurized-milk cheese. The raw-milk cheese developed a little more flavor than the pasteurized-milk cheese; with both types the body and texture were good.

Russell,[53] in 1895, isolated a number of organisms producing gas in milk and found, through inoculation experiments, that all of them had a marked effect on the texture of cheese, making it more or less spongy, and that the flavor invariably was poorer than that of the controls. The organisms apparently belonged to the *Escherichia-Aerobacter* group. Other early studies [54,55,56] indicated the importance of this group as a cause of gas in cheese, and more recent studies confirm it.

Yale and Marquardt [57] made coliform counts on 35 lots of cheese, taking samples of milk at setting, curd at milling, and cheese at different ages; after 3 days the cheese was cured at 50 F. Maximum coliform counts on 6 lots of cheese made from poor-quality raw milk (coliform counts 20,000 to 2,100,000 per ml) ranged from 9,900,000

to 410,000,000 per g and the organisms survived 6 to 12 months. With better milk containing fewer coliform organisms, they survived 3 to 6 months. The pasteurized milk used contained <1 coliform organism per ml, but coliform counts on cheese curd ranged from 900 to 57,000 per g because of recontamination and growth, even with special precautions. Acid development to *p*H 5.0, or less, greatly inhibited growth of coliform organisms but resulted in cheese with acid defects.

Escherichia-Aerobacter organisms in the milk received at cheese plants come from the usual sources although in at least two cases [54,56] they appear to have come from the interior of the udder. With large numbers in the raw milk, they may survive normal pasteurization and in certain outbreaks of gas in cheese the defect has been controlled only by improving the milk supply so that it contained fewer *Escherichia-Aerobacter* organisms. Serious recontamination of pasteurized milk is a frequent occurrence, especially in warm weather. When a vat is used a second time in a day's operations, it should be washed and sanitized before the second lot of milk is added since, because of their growth, even small numbers of organisms carried over from the first lot of cheese may cause difficulty. In some cases the lactic culture used has been contaminated with *Escherichia-Aerobacter* species.

Experience has shown that gas holes may form in cheese cured at relatively high temperature when they do not at a lower curing temperature. Presumably, at the higher temperature the gas is formed more rapidly than it can diffuse from the cheese.

Where legal, gas formation in various cheeses sometimes has been controlled by adding such chemicals as potassium nitrate or potassium chlorate to the milk. Gas formation by different organisms is limited or prevented by these chemicals which act as hydrogen acceptors and probably in other ways.

Openness in Cheese. In some cases cheese shows angular openings that often are more numerous and larger than the normal mechanical openings. Such openness in cheese often is criticized. Presumably, various causes are involved, including bacterial action.

Sherwood [57] found that "open" cheddar cheese evolved carbon dioxide much more rapidly than "close" cheese. Lactobacilli and betacocci capable of producing carbon dioxide relatively rapidly could be isolated from open cheese, and addition of such organisms to milk for cheese resulted in development of slit openness in the cheese. The most common types causing slit openness appeared to be gas-

producing lactobacilli. Overcast and Albrecht [58] isolated several cultures of *S. citrovorus* from cheese showing slit openness. Two commercial lactic cultures produced the defect when the cheese was ripened at 50 to 58 F. With an *S. lactis* culture there was little or no evidence of it.

Cheese made so that it has a very firm body may favor development of openness by delaying diffusion of the gas normally produced.

Off Flavors in Cheese. Various off flavors are encountered in cheese, and a number of them are caused by organisms. Composition of the cheese may be a factor with certain of them; for example, some off flavors due to organisms are more common when the moisture content is high and/or the acidity low.

Bitterness. Bitterness in cheese varies from only a slight defect to one that is very conspicuous.

In an outbreak of bitterness in cheese described by Harrison,[59] a bitter condition occasionally was noted in the milk as it was dumped into the weigh tank but was more evident when the milk was warmed for setting and increased as the curd was heated. When the curd was very bitter, the whey showed small gas bubbles, especially on heating. There were variations from day to day in the bitterness and it sometimes was accompanied by a fruity flavor. A yeast that produced a bitter taste in milk was isolated from samples of bitter curd. Of 99 samples of milk from as many patrons, 97 contained yeasts, several species often being present; the predominant species, the one producing bitterness, was named *Torula amara*. The organism was isolated from can washings and whey and also was found on the leaves of certain maples. Ten min at 143.6 F killed it. Gas was produced in milk. In 5 or 6 hr at 98.6 F inoculated milk had a bitter flavor. Butter made from cream into which the organism had been inoculated showed a pronounced, bitter, disagreeable flavor.

A bitter flavor in cheese has been ascribed to *S. paracitrovorus* [60] and to acidoproteolytic cocci; *S. liquefaciens* also appears to be of importance. Presumably, bitter products of various types can be produced in cheese by a variety of organisms.

Variations in the making process also may cause bitterness in cheese. Phillips [15] found with various lactic cultures that, when the *p*H of the cheese was 4.90 at 4 days, bitter flavor subsequently developed; it appeared in a majority of the cheese with a *p*H of 4.95, in part of the cheese with a *p*H of 5.00, but not in cheese of higher *p*H. Within the limits of salt contents used, no relationship was found between bitter-

ness and salt content. Price [61] noted that bitter flavor in cheese seemed to be associated with development of a slightly excessive acidity.

Unclean Flavors. Apparently, a variety of organisms can cause unclean flavors in cheese, with *Escherichia-Aerobacter* types of special importance. Whitehead [62] reported that representative *Escherichia-Aerobacter* organisms produced unclean or cowy flavors in cheese when added to pasteurized milk immediately before manufacture of the cheese. They did not produce gas holes even when present in large numbers, provided an active lactic culture was used in the milk. It was suggested that metabolism of the organisms was modified by the lactic acid streptococci so that, even with growth, little or no gas was produced.

Rancidity. Although various lipolytic bacteria are sensitive to acid, organisms of this general group sometimes produce rancidity in cheese. The defect also is caused by milk lipase.

In a study of rancid flavors in cheese, Hood and White [63] found that introduction of undesirable bacteria into the milk produced the defect. They concluded that bacteria which produce rancid flavors in cheese may come from poor-quality milk, improperly sterilized equipment, poor lactic culture, milk cans used to return unpasteurized whey to farms, contaminated water, and insanitary conditions in the factory. In later studies by Hood *et al.*,[64, 65] addition of lipolytic organisms to milk for cheese resulted in lower scores for the cheese, the chief defects being unclean and rancid flavors. The defects were evident in 14 days and increased with longer holding.

Hlynka *et al.*[66] produced rancid and other less defined flavors in cheese by addition of commercial lipase to the milk. With added lipase a higher flavor score was obtained in cheese when larger amounts of pepsin and/or rennet were used in the manufacture than with smaller amounts; this suggests that proteolytic enzymes may enhance cheese flavor by preventing rancidity due to lipase. In whey-fat mixtures prepared by homogenization, Gould [67] found that rennet extract in amounts from 3 to 20 oz per 1,000 lb, when used to precipitate the casein or when added directly to rennet whey, did not exhibit marked inhibitory effects on lipase activity. Hlynka *et al.*[68] reported that rancid and unclean flavors in cheese were fundamentally related and were caused by activity of milk lipase. The lipase was believed to be activated by vigorous agitation of uncooled cheese milk. The longer the agitation, the lower the score of the resulting cheese; the lower the temperature at which the milk was agitated, the

better the flavor score. The results suggest that minimum agitation together with cooling are desirable in producing milk for cheese. Later,[69] it was noted that milk which was churned for 15 min at 86 F showed fat dispersion roughly equivalent to that in milk containing 20% homogenized milk.

Metallic and Oxidized Flavor. Cheese having a metallic and oxidized flavor was found by Stine *et al.*[70] to have a copper content above 2 ppm. Factories having all stainless steel equipment produced cheese with 0.5 to 1.5 ppm copper and factories having tinned copper equipment produced cheese with 0.5 to 33.8 ppm copper, depending on the copper exposed. Copper in cheese above 2 ppm prevented or retarded development of normal cheddar flavor, and in some cases a metallic or oxidized flavor appeared. The copper present in milk for cheese largely was concentrated in the cheese, which had from 7 to 10 times the copper content of the milk.

Miscellaneous Flavors. Because of their acid tolerance, various yeasts grow in cheese and may produce flavor defects. Harding *et al.*[71] investigated a class of off flavor designated as "sweet" and concluded it was due to yeasts. Cheese of normal flavor contained very few if any yeasts, whereas cheese showing the sweet flavor contained large numbers, at least during the first stages of ripening. Addition of cultures of certain yeasts to milk repeatedly caused off flavors in the cheese, some of which were included under the general term of sweet flavor.

Three yeast cultures producing fruity flavor in milk were used by Hood *et al.*[72] in making experimental cheese. When the milk contained large numbers of yeasts (1,300,000 to 5,500,000 per ml) at setting, the cheese was fruity at the first grading (12 to 28 days); when it contained smaller numbers (2,100 to 340,000 per ml), 5 of 6 lots of cheese were unclean and 1 fruity at the first grading. All lots were fruity at the second grading (32 to 114 days). A culture of *E. coli* var. *neapolitana* also produced fruity flavor in cheese.

A malty flavor due to organisms is encountered in cheese and may be pronounced. Putrefactive flavors result from certain types of protein decomposition.

Color Changes in Cheese. Various color irregularities have been noted in cheese and, in some cases, organisms are responsible.

Rusty Spot. Harding and Smith[73] studied development of red or rust-colored spots in cheese made in New York and found the causative organism was one which had been isolated in Canada.[74] The spots were very fine and quite evenly distributed throughout the

cheese although they were most noticeable on the surfaces of gas holes and other openings; from 4 to 8 days usually were required for development. Texture and flavor did not appear to be influenced. Although cheese made from milk of individual patrons indicated that at times the milk coming to a plant contained the organism, the main source appeared to be factory contamination. Thorough steaming of utensils and equipment, together with good factory sanitation, brought the defect under control. Return of whey to the farms may have been a means of scattering the organism in the barn surroundings. Davis and Mattick [75] also isolated the organism causing rusty spot in cheddar cheese. They found it to be an anaerobe that formed pigment only under certain conditions and regarded it as one of the true lactic acid bacteria that produces pigment under favorable influences. Later,[76] it was noted that the most important factor in growth of the organism in milk is the growth of certain other bacteria. The organisms most active in metabiotic stimulation of pigment production were those inducing low oxidation-reduction potentials in milk. Pederson and Breed [77] reported that rusty spot in cheese is caused by chromogenic varieties of at least two distinct lactobacilli, namely, *L. plantarum* var. *rudensis* and *L. brevis* var. *rudensis*. It was pointed out that the two species belong to the two main groups in the genus *Lactobacillus;* the first forms only a trace of carbon dioxide in its production of lactic acid from glucose and other carbohydrates, whereas the second produces, in addition to lactic acid, carbon dioxide, alcohol, and volatile acids from glucose, and mannitol from levulose.

Rusty spot was encountered in herrgard cheese by Thome and Lindgren [78] and ascribed to an organism closely resembling *L. plantarum* var. *rudensis*. The spots appeared in the cheese in about 1 month. Presence of lactose and a low oxidation-reduction potential appeared to encourage pigment production.

Special Color Defects. Hood and White [79] described an unusual color in Canadian cheese shipped to England. When cut, or bored, the defective cheese exhibited varied intensities of color ranging from normal to pink, reddish, brown, and muddy shades; uncolored cheese showed dirty brown, muddy shades. Chemical tests indicated the presence of nitrates or nitrites in the cheese and, on bacteriological examination, nitrate-reducing organisms capable of producing color changes in annatto nitrate milk were isolated. The typical color defect developed in cheese made from milk inoculated with mixed cultures of organisms when potassium nitrate was added to the salt used. The defect appeared in 7 weeks, whereas in cheese made from milk in-

oculated with the organisms but not treated with potassium nitrate the color still was normal after 3 months. The nitrate apparently was added to cheese in certain Canadian factories to prevent objectionable fermentations. Harrison [80] also isolated nitrate-reducing organisms from the discolored cheese. *Ps. fluorescens* is a water organism capable of producing discoloration in milk with added annatto and potassium nitrate.

Cheese that showed light brown to yellowish spots on a freshly cut surface was studied by Hood and White.[81] The spots varied in size from a pin head to 0.5 in. in diameter; many of them contained a hard black core and the centers of others were smudgy black. After bacteriological examinations showed no unusual flora, microscopic study revealed small pieces of wire in many of the discolored areas. They evidently came from steel wool used in cleaning the cheese vats.

Changes in the pigments of annatto may be involved in certain color changes in cheese. Barnicoat [82] reported that in development of bleached and pink discoloration in cheese an oxidation may be involved. Later,[83] he noted that pink discoloration in cheese can be produced by removal of some of the yellow component of annatto, since it was shown that (1) annatto acts as an indicator, being reddish in acid solution, (2) it tends to precipitate in acid solution, (3) the acid-precipitated pigment on the casein of cheese is more red than the same strength dye in alkaline solution, and (4) oxidation preferentially destroys the more yellowish, unprecipitated fraction, leaving the more red precipitated dye, which gives a pink appearance to cheese.

Molds on Cheese. Cheese provides satisfactory nutrients for molds and, since mold spores are so widely distributed, there is opportunity for the contamination of cheese during making. When taken from the press, cheese commonly are dried for a time and then waxed, using the wax at a high temperature. Wax excludes air so molds commonly do not grow, but breaks or defects in the wax, irregularities in the cheese surface, gas escaping from the cheese, etc., sometimes permit mold growth to start. This, in turn, may force the wax away from the cheese surface and increase the air supply. Instead of being waxed, cheese sometimes are made in blocks and wrapped in sealed flexible films.

Although mold growth normally starts at the surface of cheese, when once begun it may progress well into the cheese through openings in it. When a cheese is plugged, molds often develop around the returned plug, unless it is properly sealed.

Mold Inhibitors. Use of mold inhibitors has been considered in connection with cheese, especially pieces cut for sale, since mold growth on them often is very troublesome. Miller [84] reported studies on mold inhibition on cheese cuts wrapped in moisture-proof coverings. Immersion of cuts in 8% propionic acid solutions increased the mold-free life at 60 F from the usual 3 to 5 days to 12 to 38 days, depending on the cheese and method of treatment. Solutions of 14 to 16% calcium or sodium salts were required for similar effects. Extent of contamination, period of immersion, and period of draining all influenced the results. Concentrations of propionic acid up to 40% did not entirely eliminate mold growth. Ingle [85] studied the effect of propionates on cuts of natural cheese wrapped in tinfoil, moisture-proof cellophane, or pliofilm, the cheese being dipped in propionate solutions or the wraps being sponged with them. Samples dipped in 8% propionic acid remained free of visible mold about twice as long as the controls; with 8% calcium or sodium propionate, the samples kept somewhat better than the controls but did not approach those treated with propionic acid. Sponging the wraps had little effect in holding down surface mold.

Figure 45. Mold invasion through cracked coating on a longhorn-style cheddar cheese.

Wolf [86] tested cheese wraps which had 2% dehydroacetic acid added to the wax coating used on the paper. The wrapped cheese was held at room temperature and molding was conspicuously delayed. After tests indicated that dehydroacetic acid and its sodium salt had a fungistatic action on several species of molds, Brunner and Mallmann [87] wrapped cheese in untreated parchment, in wet or dry parchment impregnated with sodium dehydroacetate, or in wet parchment that was impregnated and dipped in a 1% solution of the salt. The untreated samples showed surface mold after 14 days, whereas the dipped samples showed little or no mold after 56 days; the other two classes were intermediate. Cheese coated with paraffin wax contain-

ing 0.5% sodium dehydroacetate was free from mold after 56 days, whereas cheese coated with paraffin wax alone showed mold growth where the wax was cracked.

Sorbic acid has been investigated extensively as a mold inhibitor. Smith and Rollin [88] reported that mold growth on small packages of cheese could be controlled by treating the thermoplastic-coated cellophane wraps with 2.5 to 5.0 g sorbic acid per 1,000 sq in. and no objectionable taste, odor, or color developed. Mold growth was effectively inhibited [89] only (1) when the treated wraps were sealed to prevent recontamination, (2) when the wrap and cheese were in close contact so as to produce quickly a high concentration of sorbic acid on the cheese surface, and (3) when initial mold contamination was relatively light. Melnick and Luckmann [90] noted that sorbic acid migrated rapidly from the wraps so that soon only a small percentage of the sorbic acid remained on them. Total recovery of the acid from cheese was obtained only when large quantities were applied to the wraps. With suboptimal amounts, a high proportion of it tended to disappear from the cheese. Melnick *et al.*[91] showed that metabolic oxidation by molds was responsible for disappearance of sorbic acid from cheese in treated wraps. It was considered that inhibition of the dehydrogenase system in molds by relatively high concentrations of sorbic acid is responsible for the fungistatic, or occasionally fungicidal, activity of the acid.

Diets containing up to 5% sorbic acid (dry basis) were found by Deuel *et al.*[92] to be completely harmless to rats and dogs. Evidence obtained with rats [93] indicated that the intermediary metabolism of sorbic acid is identical with that of the naturally occurring fatty acids, caproic and caprylic, and that, under normal conditions, sorbic acid is completely oxidized to carbon dioxide and water.

Mottern [94] prevented mold growth on sliced process and natural cheese for periods up to 12 weeks by wrapping in parakote-type wraps containing 0.3 to 0.45% dimethyl dichlorosuccinate. Cheese in untreated wraps showed mold in 22 to 32% of the samples after 12 weeks, as compared with 1 to 4% in treated wraps. The maximum amount of dimethyl dichlorosuccinate transferred from the wraps to a 0.5-lb package of cheese was 2.5 mg. Although toxicity tests on rats and mice showed the compound to be several times as toxic as benzoic or sorbic acid, it was considered perfectly safe because of the low concentrations required and the low transfer rate to the cheese.

In laboratory tests Trickel *et al.*[95] found that methyl bromide gas kills molds which sometimes attack the surface of 1-lb or 0.5-lb pack-

ages of cheese. Ten lb methyl bromide per 1,000 cu ft of air prevented molds from growing after a 4-hr exposure; 2 lb required an exposure of 14 hr. The temperature had to be higher than 55 F for the gas to kill the molds effectively. The cheese can be packed before gas treatment because gases can penetrate some of the common food packaging films.

Various foreign investigators have studied the control of molds on cheese. Pedersen *et al.*[96] found that sodium benzoate (15%) in brine and nipagin (4%) in alcohol or oil inhibited mold growth on cheese but damaged the rind and caused off flavors in the cheese. Mold growth was prevented on parchment used for packing finished cheese when the above chemicals were incorporated into the parchment. Hendrickk and De Vleeschauwer[97] compared concentrations of 5 to 20% sodium nipagin, sodium benzoate, sodium salicylate, and sodium alginate in aqueous solution, and benzoic and salicylic acid in mineral oil, for their ability to prevent mold growth on cheese rind. Of these materials, only sodium nipagin in concentrations of 15 to 20% prevented mold growth for 6 to 7 weeks. The investigators[98] also conducted trials in which cheese (7 to 9 days old) were surface treated with aqueous solutions of sodium nipagin (15%), nipacombin A (10 to 20%), and vitamin K_5 (20 to 300 ppm). After treatment the cheese were stored at 50 F and relative humidities of 90 or 92%, or 84 to 86%, for 6 weeks. Little mold growth occurred after 6 weeks on cheese treated with sodium nipagin or nipacombin A, but those treated with vitamin K_5 showed extensive mold. In all cases the cheese stored at the lower humidity showed less mold.

Tammisto and Peltola[99] found that edam cheese treated with a 15% aqueous solution of sodium nipagin M before curing in the warm room and then paraffined after curing did not develop mold growth or other defects. Thome[100] reported that hard cheeses dipped in paraffin wax containing 3% ethyl *p*-nitrobenzoate showed significantly less surface mold after 10 to 45 days at 53.6 F than comparable cheeses dipped in paraffin wax alone.

Smith[101] found that ultraviolet light emitted by a 15-watt, low-pressure, mercury-vapor lamp did not prove effective in decreasing air-borne mold spores in a cheese curing room. Direct irradiation failed to prevent mold growth on the surface of the cheese.

Clarification of Milk for Cheddar Cheese

Removal of most of the sediment and some of the bacteria from market milk by clarification suggested use of the process with milk

to be made into cheese. Fisk and Price [102] found that, regardless of the milk quality, clarification improved the quality of cheese, and the cheese kept better in storage. Combs *et al.*[103] concluded that clarification of milk of poor quality under ordinary plant conditions gave no increase in the score of cheese, but with extreme care in the plant there was an increase of 1.59 points; with milk of high quality, clarification under ordinary plant conditions resulted in an increase of 2.53, and with extreme care in the plant there was an increase of 2.83.

After obtaining some improvement in cheese by clarifying the milk used, Wilster [104] studied, in 6 trials, the influence of adding clarifier slime obtained from 40 gal of milk to 20 gal of clarified milk intended for cheese, using unclarified milk as controls. The slime caused a decrease of 1.08 points in the total score in 1-month-old cheese and of 1.25 in 3-month-old cheese.

Hood and Hlynka [105] noted that clarification of milk for cheese resulted in (1) removal of sediment, (2) improvement in flavor and texture of cheese, and (3) reduction in fat loss in whey. Clarified milk developed acid faster and was ready to set earlier than unclarified milk.

Attempts to Accelerate Ripening of Cheddar Cheese

Although some cheese are used very young, with little attention being given to the flavor, others are ripened for considerable periods. Various attempts have been made to develop more desirable flavors during ripening and also to reduce the ripening period.

Special Bacterial Cultures. The importance of bacteria in cheese ripening and the difficulty in establishing the particular role of different species have led to tests in which organisms other than the lactic culture species and lactobacilli have been added to milk for cheese.

Hansen [106] noted that addition of 0.05% of a milk culture of *S. liquefaciens,* or 0.5% of a milk culture of an unidentified *Micrococcus,* to pasteurized milk improved the flavor of cheese made from it but did not materially influence the nitrogenous decomposition; a lactic culture and *L. casei* also were added to the milk. *Ps. fluorescens, Alc. viscosus,* and *Ach. lipolyticum* had little effect on the flavor of cheese but *Ps. fragi* decreased the flavor score. Other investigations [107] in which *S. liquefaciens* was used have resulted in pronounced bitterness in the cheese, perhaps because of the strain used or the amount of inoculation. The general action of this species in milk suggests that extended action in cheese would be undesirable.

Harris and Hammer [108] found that 34 *Micrococcus* cultures, origi-

nally isolated from cheddar cheese, had the following effects on the flavor of cheese when inoculated into pasteurized milk used for it: 7 had undesirable effects, producing bitter and unnatural flavors; 14 had no effect; and 13 had desirable effects. Strains apparently belonging to the same species differed in their effects. Following their observation [109] that in raw-milk cheese which developed a good flavor micrococci were the predominant nonlactic organisms in the early stages of ripening, Alford and Frazier [110] found that selected strains of *M. freudenreichii* increased the rate of flavor development during the early months of ripening in pasteurized-milk cheese. Some of the strains reached their maximum numbers by the time the cheese was in the press and then decreased slowly for several months, while others showed a similar increase and then rapidly decreased in numbers.

When used in small numbers,[108] certain strains of propionic acid bacteria improved the flavor of cheddar cheese, whereas others did not; in large numbers all the strains improved the flavor and some produced a distinct sweet flavor somewhat resembling the flavor of swiss cheese. Cheddar cheese appears to contain propionic acid organisms rather commonly. Deane *et al.*[111] added various organisms to milk intended for cheese and noted an inconsistency in the results. The most promising results were obtained with an acidoproteolytic organism (isolated from old cheese) and a lactic culture.

Dahlberg and Kosikowsky [112] isolated a strain of *S. faecalis* that rapidly fermented lactose. When used in milk for cheese it produced acid somewhat slower than a lactic culture but fast enough for cheese making. Cheese made with it had more cheddar flavor, and the body was more mellow and waxy than in cheese made with lactic culture. The best flavor was obtained when both cultures were used. *S. faecalis* [113] grew and survived in cheese for considerable periods at both 50 and 60 F. Cheese made in a commercial plant [114] from pasteurized milk which naturally contained bacteria that ripened the cheese especially well was improved slightly, or not at all, by use of *S. faecalis*. In studies on the safety of using a specific strain of *S. faecalis* as a culture for cheese, Dack *et al.*[115] found that cheese made with the culture and containing many viable organisms and appreciable quantities of tyramine was without effect when fed to human volunteers.

Higher Curing Temperatures. With cheese made from raw milk, curing temperatures often are kept relatively low to limit action of organisms that may cause objectionable conditions. Use of pasteurized milk partly overcomes this hazard and permits attempts to speed

up the ripening by use of higher temperatures. However, with underheated or recontaminated pasteurized milk, high curing temperatures for the cheese may result in rapid development of defects.

Riddet *et al.*[24] reported that the effect of holding cheese at 60 F, compared to 45 to 50 F, was evident in the increase in soluble nitrogen values and in the more rapid maturing of the cheese. It appeared that an extra 2 weeks at 60 F during the early stages of ripening was more effective than holding an extra 6 weeks at 45 to 50 F subsequent to the normal 2 weeks in the factory curing rooms at 60 F. All the cheese held at the higher temperature had more open texture but good cheese closed up to some extent as it matured. Freeman and Dahle [26] noted that proteolysis in cheese ripening could be increased 40 to 100% by raising the ripening temperature from 45 to 63 F; the maximum flavor score was reached more quickly at 63 F and was as high as at the lower temperature.

In studying the effect of ripening temperature on the quality of short-cure Texas cheese, Hanson *et al.*[25] found that 60 F gave the most desirable results and that up to 70 F gave satisfactory results. Rate of proteolysis was increased by increasing the temperature; proteolysis could be checked by moving the cheese to a lower temperature. According to Deane *et al.*[111] a preincubation at 63 to 65 F for 4 weeks followed by curing at 43 to 45 F usually induced a maximum flavor score sooner than continuous curing at 43 to 45 F, but the cheese did not always maintain as high a flavor score. Sanders *et al.*[116] found cheese made from pasteurized milk of good quality was as fully ripened in 3 to 4 months at 60 F as that cured 6 months at 50 F and developed better flavor.

Use of high curing temperatures presents some problems. Erekson [117] noted that curing cheese below 40 F affords the following advantages compared to curing at 60 F: (1) There is no loss from insects, (2) shrinkage is kept at a minimum, (3) special humidity control in commercial storage is seldom necessary, occasional circulation of air being sufficient, (4) maximum use of storage space is obtained, and (5) cheese can be held 1 to 2 yr without handling and with a minimum selection of original stock and minimum deterioration in grade. He listed the advantages of high-temperature curing as (1) cheese with a special sharp flavor can be produced, and (2) the flavor can be produced in 3 to 4 months with unpasteurized-milk cheese. A high flavor also can be obtained with pasteurized-milk cheese, but it is not comparable to that of raw-milk cheese.

Special Proteolytic Enzymes. Use of special proteolytic enzymes in cheese manufacture offers some interesting possibilities from the standpoint of accelerated ripening. Freeman and Dahle [26] reported that addition of pure rennin increased the rate of proteolysis in cheese ripening, increased slightly the rate at which flavor developed, and produced an aged product with a slightly higher flavor score. Added pepsin also increased the rate of proteolysis, particularly at the beginning of ripening and at lower ripening temperatures. It did not accelerate development of flavor but produced an aged product with appreciably higher score. Added trypsin increased markedly the rate of proteolysis during the early part of ripening, after which its effect was greatly reduced; it also increased the rate at which flavor developed but reduced the maximum flavor score.

Babel *et al.*[118] obtained a cheese with a soft waxy body by using an enzyme preparation from chicken proventriculi to coagulate the milk and then following a modified edam process in the making. The active proteolysis was accompanied by development of a bitter flavor, which later tended to disappear.

Increasing Fat Hydrolysis. There have been various suggestions that fat hydrolysis plays a part in flavor development in cheddar cheese. Early studies [39] indicated the possibility of fat hydrolysis accounting for certain fatty acids obtained from cheese. Lane and Hammer [119] noted that in aged cheese considerable flavor suggestive of ripened cheese was contained in the fat. Acid numbers of fat from fresh (2-day) cheese were relatively high, compared to those of fat from sweet-cream butter, and increased with ripening; values for raw-milk cheese were higher when the cheese was fresh and increased more rapidly than those for pasteurized-milk cheese. Volatile acids of raw-milk cheese regularly were higher than those of pasteurized-milk cheese during the 200 days of ripening; values for the former usually decreased slightly after 90 days, whereas those for the latter continued to increase. Cheese [120] made from raw homogenized cream mixed with skim milk (raw or pasteurized) commonly developed a rancid flavor early in the ripening, but the condition tended to disappear as ripening progressed and, eventually, the cheese was not rancid and was better in flavor than cheese made from pasteurized milk or pasteurized homogenized cream mixed with pasteurized skim milk. Cheese made from pasteurized milk had low fat acidities and lacked flavor, whereas cheese having higher fat acidities commonly had more flavor. Addition of pancreatin to pasteurized milk for cheese resulted in a disagreeable rancid flavor immediately after manufacture and the

condition did not disappear during ripening; often the ripened cheese was bitter. Desiccated mammary tissues, or extracts of them, were lipolytic and their addition to pasteurized milk commonly had a desirable effect on the cheese made from it. Early in the ripening the tissues or extracts, particularly in the larger amounts, commonly caused rancidity in the cheese, but this disappeared as the ripening continued and after some time the flavor generally was better than the flavor of cheese made from pasteurized milk without the addition. The mammary tissues or extracts increased the fat acidity of the cheese.

Babel and Hammer [121] found in trials with pasteurized milk that use of rennet paste resulted in an increase in fat acidity of the cheese. Some of the cheese showed rancidity early in the ripening but this disappeared, or nearly so, when the cheese was 6 months old. Cheese that was not rancid was superior in flavor to the control cheese. Use of a small amount of rennet paste tended to mask the acid flavor in the control cheese.

The relationship of organisms to the disappearance of rancidity produced in cheese with various procedures was studied by Cherrington and Hammer.[122] An organism, which appeared to be a variant of *S. lactis*, developed regularly in 3 to 4 days in cheese made rancid by adding butyric acid to the milk used. As the cheese ripened the rancidity disappeared and the organism decreased in numbers. The disappearance of rancidity in cheese appeared to be due to activity of organisms normally in cheese which apparently changed their morphologic and biochemical characteristics.

The general evidence suggests that fat hydrolysis has an influence on the flavor of cheese and that one of the differences between raw-milk and pasteurized-milk cheese is the absence of milk lipase action in the latter. It appears that in ripening of cheese made from raw milk the products of lipolysis, which would give a rancid flavor if they accumulated, commonly are destroyed and that the mechanism even may cause the disappearance of a rancid flavor after it once is evident, provided it is not too conspicuous. Trials in which various lipases were added to pasteurized milk for cheese have not yielded the desired results; presumably, the enzyme preparations caused changes that were not satisfactory from the standpoint of flavor development. The use of heat exposures below those giving definite pasteurization in attempts to speed up flavor development in cheese may involve, in part, preservation of lipase activity. The importance of milk fat as a source of flavor in cheese is indicated by the lack of

typical cheddar flavor in filled cheese, in which the milk fat is replaced by vegetable fat.

REFERENCES

General and Cheddar Cheese

1. *U. S. Dept. Agr. Handbook,* 54, 1953.
2. *Wis. Agr. Expt. Sta. Research Bull.* 25 (1912).
3. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 87 (1921).
4. *J. Dept. Agr. Victoria,* **23,** 421 (1925).
5. *J. Dairy Sci.,* **28,** 597 (1945).
6. *Wis. Agr. Expt. Sta., 18th Ann. Rept.,* 162 (1901).
7. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 201 (1932).
8. *Ont. Agr. Coll. Bull.* 130, 1903.
9. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 8 (1908).
10. *J. Dairy Research,* **8,** 74 (1937).
11. *J. Dairy Sci.,* **30,** 231 (1947).
12. *J. Dairy Sci.,* **24,** 969 (1941).
13. *J. Dairy Research,* **5,** 185 (1934).
14. *Ia. Agr. Expt. Sta. Research Bull.* 190 (1935).
15. *J. Dairy Sci.,* **18,** 471 (1935).
16. *J. Dairy Research,* **8,** 224 (1937).
17. *J. Dairy Research,* **10,** 426 (1939).
18. *J. Dairy Research,* **10,** 449 (1939).
19. *J. Dept. Agr. S. Australia,* **49,** 69 (1945).
20. *Intern. Dairy Congr., Proc. 13th Congr.,* **4,** 614 (1953).
21. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 54 (1893).
22. *Wis. Agr. Expt. Sta., 17th Ann. Rept.,* 102 (1900).
23. *Helv. Chim. Acta,* **36,** 1955 (1953).
24. *New Zealand Dept. Sci. Ind. Research Bull.* 38 (1933).
25. *Texas Agr. Expt. Sta. Bull.* 646 (1944).
26. *Pa. Agr. Expt. Sta. Bull.* 362 (1938).
27. *Natl. Butter Cheese J.,* **39** (5), 42 (1948).
28. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 203 (1901).
29. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 200 (1932).
30. *J. Dairy Research,* **8,** 238 (1937).
31. *J. Dairy Sci.,* **35,** 149 (1952).
32. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 236 (1903).
33. *New Zealand Dept. Sci. Ind. Research Bull.* 37 (1933).
34. *J. Dairy Research,* **5,** 42 (1933).
35. *Food Research,* **9,** 278 (1944).
36. *J. Dairy Sci.,* **17,** 33 (1934).
37. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 270 (1943).
38. *J. Dairy Sci,* **28,** 187 (1945).
39. *Wis. Agr. Expt. Sta. Research Bull.* 11 (1910).
40. *J. Dairy Sci.,* **21,** 777 (1938).
41. *J. Dairy Sci.,* **32,** 769 (1949).
42. *J. Dairy Sci.,* **34,** 235 (1951).
43. *J. Dairy Sci.,* **31,** 293 (1948).
44. *J. Dairy Sci.,* **31,** 305 (1948).
45. *J. Dairy Sci.,* **32,** 515 (1949).
46. *J. Dairy Sci.,* **32,** 521 (1949).
47. *J. Dairy Sci.,* **25,** 31 (1942).
48. *J. Dairy Sci.,* **21,** 161 (1938).
49. *J. Dairy Research,* **10,** 118 (1939).
50. *J. Dairy Sci.,* **36,** 368 (1953).
51. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 231 (1903).
52. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 266 (1942).
53. *Wis. Agr. Expt. Sta. 12th Ann. Rept.* 139 (1895).
54. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 158 (1899).
55. *Mich. Agr. Expt. Sta. Bull.* 183 (1900).
56. *Ont. Agr. Coll. Bull.* 141 (1905).
57. *J. Dairy Research,* **10,** 326 (1939).

58. *J. Dairy Sci.*, **35**, 554 (1952).
59. *Ont. Agr. Coll. Bull.* 120 (1902).
60. *J. Dairy Sci.*, **16**, 121 (1933).
61. *J. Dairy Sci.*, **19**, 185 (1936).
62. *J. Dairy Research*, **2**, 76 (1930).
63. *Can. Dept. Agr. Bull.* 146 (1931).
64. *Can. Dairy Ice Cream J.*, **28** (2), 27 (1949).
65. *Can. Dairy Ice Cream J.*, **28** (6), 27 (1949).
66. *J. Dairy Sci.*, **24**, 561 (1941).
67. *Mich. Agr. Expt. Sta. Quart. Bull.*, **26**, 75 (1943).
68. *J. Dairy Sci.*, **26**, 1111 (1943).
69. *J. Dairy Sci.*, **28**, 79 (1945).
70. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 350 (1949).
71. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 183 (1900).
72. *Sci. Agr.*, **32**, 638 (1952).
73. *N. Y. (Geneva) Agr. Expt. Sta. Bull.* 225 (1902).
74. *Can. Dept. Agr. Bull.*, 1897.
75. *J. Dairy Research*, **1**, 50 (1929).
76. *J. Dairy Research*, **2**, 190 (1931).
77. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 259 (1941).
78. *Medd. Statens Mejeriforsok*, No. 31 (1951).
79. *Can. Dept. Agr. Bull.* 128 (1929).
80. *Can. J. Research*, **1**, 256 (1929).
81. *Sci. Agr.*, **10**, 520 (1930).
82. *J. Dairy Research*, **8**, 53 (1937).
83. *J. Dairy Research*, **14**, 59 (1945).
84. *Proc. Food Conf. Inst. Food Technol.*, **1**, 153 (1940).
85. *J. Dairy Sci.*, **23**, 509 (1940).
86. *Food Technol.*, **4**, 294 (1950).
87. *Mich. Agr. Expt. Sta. Quart. Bull.*, **33**, 127 (1950).
88. *Food Research*, **19**, 59 (1954).
89. *Food Technol.*, **8**, 133 (1954).
90. *Food Research*, **19**, 28 (1954).
91. *Food Research*, **19**, 44 (1954).
92. *Food Research*, **19**, 1 (1954).
93. *Food Research*, **19**, 13 (1954).
94. *Food Eng.*, **26** (10), 93 (1954).
95. *Wis. Agr. Expt. Sta. Ann. Rept. Bull.* 510 (1954).
96. *Beretn. Forsøgsm. Kbh.*, 70 (1951)
97. *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent.*, **16**, 287 (1951).
98. *Mededel. Landbouwhogeschool en Opzoekingsstas. Staat Gent.*, **17**, 669 (1953).
99. *Karjantuotteesta.*, No. 12, 303 (1948).
100. *Svenska Mejeritidn.*, **42**, 557 (1950).
101. *J. Dairy Sci.*, **25**, 525 (1942).
102. *N. Y. (Cornell) Agr. Expt. Sta. Bull.* 418 (1923).
103. *J. Dairy Sci.*, **7**, 524 (1924).
104. *Ia. State Coll. J. Sci.*, **4**, 181 (1930).
105. *Natl. Butter Cheese J.*, **37** (1), 38 (1946).
106. *B. W. Hammer Panegyric*, 123 (1937). Collegiate Press, Inc., Ames, Ia.
107. *J. Dairy Sci.*, **34**, 776 (1951).
108. *J. Dairy Sci.*, **23**, 701 (1940).
109. *J. Dairy Sci.*, **33**, 107 (1950).
110. *J. Dairy Sci.*, **33**, 115 (1950).
111. *Natl. Butter Cheese J.*, **36** (1), 25 (1945).
112. *J. Dairy Sci.*, **31**, 275 (1948).
113. *J. Dairy Sci.*, **31**, 285 (1948).
114. *J. Dairy Sci.*, **32**, 630 (1949).
115. *J. Infectious Diseases*, **85**, 131 (1949).
116. *U. S. Dept. Agr. Bur. Dairy Ind., Mimeo. Inf.* 29 (1946).
117. *Food Ind.*, **18**, 1177 (1946).
118. *J. Dairy Sci.*, **26**, 331 (1943).
119. *Ia. State Coll. J. Sci.*, **13**, 149 (1937).
120. *Ia. Agr. Expt. Sta. Research Bull.* 291 (1941).
121. *J. Dairy Sci.*, **28**, 201 (1945).
122. *Idaho Agr. Expt. Sta. Research Bull.* 14 (1948).

SWISS OR EMMENTHALER CHEESE *

Swiss, or emmenthaler, cheese is a typical hard variety in which normal ripening apparently involves the action of various bacteria, with formation of eyes and a sweet flavor. Experience and laboratory investigations suggest that one or more types of organisms be added to the milk used for swiss cheese. These include (1) a propionic acid organism (genus *Propionibacterium*) to aid in development of the desired flavor and eye formation, (2) S. *thermophilus* to improve the general quality, and (3) a *Lactobacillus* to limit abnormal fermentation and aid in controlling ripening. The various cultures are propagated in milk, whey, broth, etc. The propionic acid bacteria are the special organisms of swiss cheese and their presence in considerable numbers is so characteristic of swiss cheese that they distinguish it from all except closely related varieties.[1] From the standpoint of its manufacture, swiss cheese is characterized by a relatively high cooking temperature, such as 120 to 127.5 F; this greatly influences the flora of the cheese.

Swiss cheese commonly has been made from raw milk in the form of large wheels, using kettles for setting the milk, cooking the curd, etc. A thick rind must be developed to support the weight of a wheel (160 to 220 lb). Recently, a method using pasteurized milk and setting it and cooking the curd in vats has been perfected. The curd is made into blocks and cured, in limiting forms, under a flexible, fluidproof sealing material. With this procedure there is no appreciable rind, so that the cutting loss is negligible, and the composition is more uniform throughout the cheese mass.

Importance of Milk Quality. Relationship of the condition of the milk to the quality of swiss cheese long has been recognized, and twice-a-day delivery of milk was at one time used extensively in the industry. Rogers *et al.*[2] noted that one of the most important factors influencing the quality of swiss cheese is the bacteriological condition of the milk. With pasteurization of the milk, control of its bacteriological condition at the time of setting is simplified and satisfactory swiss cheese has been made from milk that probably would not yield such cheese in a raw condition.

Clarification of Milk. On the basis of general observations and experience, clarification of the milk has been adopted widely by swiss-

* References for each new type of cheese begin with 1, to reduce use of large numbers.

cheese factories. Matheson *et al.*[3] found the specific effects of clarification of the milk included: (1) Decrease in the tendency of the fat to form aggregates on standing, (2) removal of a large proportion of the leucocytes from mastitis milk, (3) an increase in rate of multiplication of culture organisms and improvement in results of fermentation tests, (4) increase in concentration of oxygen and decrease in carbon dioxide, (5) increase in rate at which the oxidation-reduction potential changes at 86 F, and (6) slight decrease in stability to alcohol. Effects of the process on the properties of the cheese included: (1) Marked decrease in number and increase in size and uniformity of eyes, (2) increase in firmness of the cheese and in incidence of the glaesler defect, (3) increase in rate of multiplication of culture organisms and of acid formation, (4) decrease in moisture content and yield of cheese, and (5) increase in fat loss in the whey. Clarifying the milk at a relatively low temperature with a relatively slow bowl speed diminished the effects of clarification on the milk and cheese, whereas decreasing the rate of flow through the bowl by one-half and increasing the temperature from 69.8 to 89.6 F increased the effects. Beneficial effects of clarification were especially pronounced with mastitis milk.

General Morphologic Groups of Bacteria. Eldredge and Rogers[4] studied the changes in the general morphologic groups of bacteria during ripening of swiss cheese by plating on a beef extract peptone whey agar and, with each plating, picking all the colonies from a representative plate. More than 900 cultures were studied in detail. Fermentation tests failed to divide them into distinct groups but they were sharply differentiated morphologically into cocci, short rods, and long rods. Young cheese contained short rods almost exclusively, but these decreased slowly while the long rods increased. After 6 to 8 weeks the 2 groups were present in about equal numbers, and at the end of the ripening long rods made up most of the flora. The cocci appeared in small numbers at intervals in the earlier stages of ripening. The rods included typical lactobacilli, but there were all gradations from these to cultures producing no acid in milk.

***S. thermophilus* in Cheese.** Because of the high cooking temperature used with swiss cheese, organisms that are relatively heat resistant are of special importance. Frazier *et al.*[5] found that under most conditions use of cultures containing S. *thermophilus* improved the cheese quality, especially when the methylene blue reduction time of the milk was 5 to 6 hr, or longer.[6] Improvement was marked in eye formation, texture, and flavor; many of the cheese made with S. *thermophilus*

showed a tendency to checking. *S. thermophilus* [7] usually increased during the whole process in the cheese kettle, but the increase commonly was more rapid late in the process when the temperature was high; *S. lactis* increased early but was stopped by the cooking temperature. *S. thermophilus* [8] usually began to grow within 3 or 4 hr after dipping and increased rapidly until the sixth or eighth hour, after which the increase was slow or there was a gradual decrease to the twenty-first hour. *S. thermophilus* appeared to be responsible for the rapid decrease in *p*H of the cheese during the first 7 or 8 hr; most of the cells were very large which may explain the great fermentative activity per cell. Burkey *et al.*[9] considered the essential function of *S. thermophilus* in cheese on the press to be production of acid that facilitates drainage from the interior of the cheese during the first 4 to 6 hr after dipping.

***Lactobacillus* Types.** The *Lactobacillus* cultures used in cheese have been of different types, including *L. bulgaricus, L. helveticus, L. casei,* and unidentified species. Apparently, the lactobacilli do not increase in the kettle and usually have decreased by the time of dipping, whereas on the press [8] they decrease for a time and then increase fairly rapidly. This relationship suggests that the organisms are of primary importance during the ripening process. Bassett and Slatter [10] reported that the heat resistance of lactobacilli isolated from raw-milk swiss cheese varied greatly, but the majority could withstand minimum pasteurization exposures.

Activity of Culture Organisms. Activity of the culture organisms used is important and also their heat resistance during the cooking.

Peppler and Frazier [11] found that active cultures of *S. thermophilus* and *L. helveticus* could be prepared with considerable latitude in incubation time and temperature and also that variations within limits in methods of incubation of stock cultures could be employed, with assurance that satisfactory growth of the cultures would be maintained. They [12] noted that a variety of modifications can be applied to the handling of cultures of thermoduric bacteria without lessening the activity or heat resistance. By using a better culture medium, one with added accessory substances, the heat resistance of cultures is not only assured but actually increased. Results indicate cultures of great heat resistance can be produced by improving the culture medium and/or by incubation at 104 F instead of 98.6 F. In the case of *S. thermophilus,* growth at 104 F requires a medium of highest quality and proper control of the incubation period to provide cultures of proper maturity. Mixed cultures of the film yeast *Candida krusei*

and *L. helveticus* exhibited greater heat resistance than pure cultures of the latter species; frequent transfer of the mixed cultures at 98.6 F reduced the influence of *C. krusei*. *S. thermophilus* grown with *C. krusei* was more active after heating than pure cultures of the *Streptococcus;* successive transfers of the mixed cultures did not decrease the influence of *C. krusei*. After symbiosis [13] with *C. krusei, S. thermophilus* and *L. helveticus* from areas near the surface film were more active following severe heat treatment than bacteria from the same culture but at lower levels. Influence of the film yeast extended some distance below the active growing region.

Propionic Acid Organisms. Von Freudenreich and Orla-Jensen [14] isolated propionic acid bacteria which they considered the cause of eye formation in cheese. The organisms fermented lactates with production of propionic acid, acetic acid, and carbon dioxide. Sherman [15] obtained a propionic acid organism from cheese and considered it the essential organism for production of eyes and flavor. It fermented lactates with formation of carbon dioxide and volatile acids, especially propionic and acetic acids.

The characteristic change brought about by the propionic acid organisms is the breakdown of lactic acid (or lactate) according to the reaction,

$$3CH_3 \cdot CHOH \cdot COOH \rightarrow$$
$$2CH_3 \cdot CH_2 \cdot COOH + CH_3 \cdot COOH + CO_2 + H_2O$$

The organisms produce propionic and acetic acids and carbon dioxide from various materials [16] and growth may be influenced by the activity of other species in making such materials available.

Kiuru [17] found that when racemic lactic acid produced by *L. helveticus*, D(+) lactic acid produced by *S. thermophilus*, and D(−) lactic acid produced by *Lactobacillus lactis* were fermented by *Propionibacterium peterssonii* in yeast autolysate, the same rate of fermentation was obtained with racemic and D(+) lactic acid but the fermentation was delayed with D(−) lactic acid.

Organisms of the genus *Propionibacterium* are small, gram-positive rods when grown under anaerobic conditions. A number of species are included in the genus. *Propionibacterium shermanii* has been used widely in making cheese. The propionic acid organisms apparently do not increase in the kettle [7] or on the press,[8] so that their activity is confined to the ripening period. Demeter and Janoschek [1] found that in cheese propionic acid organisms increase rapidly after salting

until, in about 70 days, they outnumber the lactic acid bacteria; later, they decrease but remain in the majority. Propionic acid organisms were found in other hard and semihard cheeses but did not exceed the lactic acid bacteria in number.

The propionic acid bacteria play a part in flavor development in cheese through production of propionic acid, which forms propionates, and in eye formation through production of carbon dioxide. However, other organisms also may produce carbon dioxide in cheese. There is no close relationship between flavor and eye production since cheese that lacks eyes may have a satisfactory flavor and cheese with eyes may lack flavor. In eye formation the balance between production of gas and its diffusion from the cheese appears to be important; this is influenced, in part, by the texture of the cheese.

Kiuru [18] found that in cheese the propionic acid fermentation raised the oxidation-reduction potential which had been lowered by the lactic acid bacteria. Ripened cheese was unhomogeneous as regards the potential, the zone below the rind being more negative than the interior.

Sources. The propionic acid organisms appear to be widely distributed. At one time their presence in cheese depended entirely on natural contamination, and they are found in milk and other materials.

Heat Resistance. Their survival of the usual cooking temperatures for cheese shows that propionic acid organisms are relatively heat resistant, and special tests also indicate this.[19]

Enumeration of Propionic Acid Organisms. The propionic acid organisms readily are enumerated and also isolated with a lactate agar in shake cultures; the colonies produced often are winged rather characteristically. A satisfactory composition for lactate agar is: [20] Peptone 2%, sodium lactate 1%, yeast extract 1%, washed agar 1.5%, and water to make 100% (*p*H 7.0).

Using a special yeast lactate agar, Demeter and Janoschek [1] lowered the oxidation-reduction potential with sodium sulfite so that, with an overlayer of the medium and a layer of sterile paraffin oil, the organisms could be grown in petri dishes. Kambar *et al.*[21] lowered the oxidation-reduction potential of an agar plating medium with 0.5% sodium thioglycollate and used an overlay of plain agar; this procedure simplified the counting of the organisms on a routine basis.

The propionic acid organisms can be identified tentatively on the basis of morphology, staining reactions, and production of catalase. The final step in identification is to determine their ability to ferment lactic acid, or lactate, under anaerobic conditions with formation of

propionic and acetic acids, the ratio ordinarily being approximately 2 to 1. In cheese making, use of *S. thermophilus* has been found to lower the ratio of propionic to acetic acid.[18]

Eye Formation. Eyes of the proper size and distribution are very desirable in cheese. Normal eyes vary in diameter from about 0.5 to 1 in. and are so spaced that there is 1 to 3 in. between them. Under abnormal conditions the eyes may be small and numerous, or large and few in number, whereas in some instances eye formation fails.

Clark [22] found that the gases in normal eyes are carbon dioxide and nitrogen; the nitrogen is from air occluded in the curd at the time of making the cheese so carbon dioxide is the only gas of significance.

Figure 46. Small size swiss-type cheese showing desirable eye formation. Iowa Agr. Exp. Sta. Res. Bul. 264.

In some instances he noted an evolution of gas characterized by the presence of hydrogen and considered it due to formation of gas from sugar; this detrimental type of gas production occurs soon after the cheese is made.

Because of the uneven distribution of bacteria in cheese, it might be assumed that the eyes form at the locations of colonies of the gas-producing organisms. However, the comparatively few eyes in a normal swiss cheese make this improbable and, moreover, the types of organisms present near the eyes appear to be the same as those at a distance. Clark [23] pointed out there is no need to assume that the gas separates as a bubble at the point where it is produced, and showed that in agar or gelatin gas diffuses considerable distances. The size of a gas bubble blown in agar was increased by growing gas-producing organisms some distance from it. Apparently, in cheese the gas separates at points that have no necessary relation to the localities where it is produced. When the curd particles were stained with

congo red by addition of this dye to the kettle just before the curd was drawn, the walls of normal eyes were stained, which led to the assumption that the eyes developed between curd grains.

Flavor Contributors. The propionates, of which calcium propionate presumably is the most significant, are important flavor contributors to swiss cheese. The salts have a sweet and somewhat bitter flavor. Babel and Hammer [24] found that addition of calcium or sodium propionate to process swiss-type cheese lacking in flavor gave the product a sweet flavor.

Amino acids have been studied extensively in connection with the flavor of cheese. Virtanen *et al.*[25] concluded that the sweet taste is due to proline which occurs in casein to the extent of 8.2% (% of total N). The flavor of hydroxyproline is similar to that of proline but, since casein contains only 1.5% (% of total N), its effect is slight. Proline liberated from casein is well retained in cheese. Cheese serum contains 9 to 19 mg proline per ml and this causes considerable sweetness. Most amino acids have little or no flavor in the concentrations in cheese, but when all are combined they have decided flavor. Arginine has an objectionable flavor and could have an unfavorable influence on cheese flavor unless it is decomposed after liberation. Free arginine was not present to any extent in cheese; a bitter flavor possibly originated from it. In the fluids in the eyes of cheese 11 to 12 months old, about two-thirds of the total amino nitrogen was in the form of free amino acids and one-third in the form of peptides and casein dissolved in the fluid. Free proline in the fluid equalled 12.4 mg per ml and free arginine 0.17 mg.

Storgårds and Hietaranta [26] considered that the amount of sour amino acids (glutamic and aspartic) in swiss cheese is, as a rule, greater than in other hard cheeses and the amount of basic amino acids less. The former increase and the latter decrease during storage of cheese.

The ability of *P. peterssonii* to decompose amino acids was noted by Kiuru.[27] In milk cultures in which lactic acid production by *L. helveticus* took place prior to the propionic acid fermentation, the ammonia content increased if the test continued long enough. When decomposition of pure amino acids was studied, the amounts of amino acids broken down (amino N converted to NH_3) were alanine 91%, aspartic acid 86%, serine 42%, arginine 38%, and cystine 15%. The propionic acid bacteria formed hydrogen sulfide from cystine and gave off characteristic flavors from various amino acids. The flavor of isoleucine resembled to some extent that of old cheese.

When grown in milk or whey, or in calcium lactate medium free of

citric acid and sugar, Tomka [28] found that two strains of propionic acid bacteria produced acetylmethylcarbinol and biacetyl. The organisms produced the highest values for the compounds in a few days and the values then remained unchanged.

Protein Decomposition in Cheese. The general agents responsible for protein decomposition in swiss cheese are lactic acid organisms and enzymes, much as in cheddar cheese, although the relationship between them may vary from that in cheddar cheese. Watson [29] noted that production of a desirable soft texture in swiss cheese was not necessarily coincident with the most extensive proteolysis and that variations in the colloidal structure of cheese, as shown by difference in binding of the water, exert an important effect on the texture.

Influence of Copper and Manganese. At one time practically all swiss cheese was made in copper kettles, now largely replaced by stainless steel. Mueller *et al.*[30] found that copper had no pronounced effect on growth and acid production by *S. thermophilus* and 3 species of lactobacilli. It decreased the growth rate of 3 *P. shermanii* cultures, but after 13 days production of volatile acid and carbon dioxide was about equal at all levels of copper used. Preliminary evidence indicated that high levels of copper (18 ppm) in cheese exerted an unfavorable influence on the ripening although satisfactory ripening occurred in cheese containing 0 to 0.5 and 7.5 to 8.0 ppm.

Demeter [31] greatly increased the growth of *L. casei* in whey by addition of traces of manganese and considered this important in making swiss cheese.

Swiss-type Cheese. A swiss-type cheese prepared in a vat from pasteurized milk with relatively low cooking temperatures, which permit use of a lactic culture for acid production, has been made in various countries, including the United States. Babel and Hammer [20] found that certain propionic acid cultures rather regularly produced excellent or good flavors in this type of cheese, but none of the cultures was consistent in producing good eyes; control cheese, in which no propionic acid organisms could be detected in 0.1 g, sometimes developed satisfactory eyes. By increasing the number of propionic acid bacteria added to the milk, there was a corresponding increase in the sweet flavor produced; with high inoculations some cheese were criticized as too sweet. When good flavor developed in the control cheese, propionic acid organisms were present in large numbers. Delaying the salting of the cheese resulted in an increase in the numbers of propionic acid organisms and in the sweet flavor but the cheese tended to flatten. Addition of propionic acid organisms to the milk

gave cheese with higher volatile acidity and higher acid number on the fat; the degree of sweet flavor was rather directly related to the amount of volatile acid present. *p*H values on the cheese at 90 days were not correlated with the quality. Cheese containing large numbers of *Escherichia-Aerobacter* organisms had numerous ragged eyes, which were more objectionable than the flavor; with large numbers of anaerobic spore formers present, the flavor of butyric acid increased as the cheese aged.

Defects in Swiss Cheese Due to Organisms

Lack of Eye Formation. Swiss cheese sometimes does not develop eyes in the normal manner. This may be due to failure of the propionic acid organisms, and perhaps other species, to produce gas in sufficient amounts or to an irregularity in the ratio between production and diffusion of gas.

Gassy Cheese. In some instances excessive amounts of gas are produced, and instead of the desired eye development the cheese shows many gas holes; often the shape of the cheese becomes abnormal. Gas formation in cheese may cause serious economic losses; it sometimes occurs soon after the cheese is made, but in other cases it is delayed. The responsible organisms are of various types.

Russell and Hastings [32] investigated an outbreak in a Wisconsin factory and found a lactose-fermenting yeast responsible. When the organism was added to milk that was made into cheese, it caused an extremely gassy condition, and the cheese was decidedly lacking in flavor. The investigators noted certain practices, at one time common, that tend to favor growth of the organism: (1) Holding the whey so that the butterfat can be skimmed off, (2) use of whole rennets soaked in sour whey, and (3) use of individual barrels, which are rarely cleaned, to hold whey for the farms.

Burri [33] reported that an excessive number of large holes in cheese and a splitting of the rind, together with an objectionable flavor, were due to large numbers of butyric acid organisms that came from silage. Beginning and ending of the abnormality coincided so definitely with beginning and ending of the silage feeding that the relationship was clear. Mösle [34] stated that ripening of milk for cheese permits lactic acid bacteria to develop as well as certain other types. Butyric acid fermentation was suppressed by addition of 0.01 to 0.1% ammonium nitrate. When the nitrate was added, cocci grew well in the whey and formed a heat-resistant inhibitive substance which was effective against certain undesirable fermentations in the cheese, including the butyric

acid fermentation. In Wisconsin the contention that feeding of grass silage is responsible for low-quality swiss cheese was investigated by Graf [35] who found that quality of the cheese bore no relationship to the amount of grass silage milk received by the factories.

The cause of an unusually large number of gassy cheese at a factory was investigated by Albus; [36] after failing to isolate an aerobic gas-forming organism, a strain of *Clostridium perfringens* responsible for the abnormal condition was obtained. Experimental cheese showed both the niszler type of gassy fermentation, which is characterized by a uniform distribution of very small holes that are detected when the cheese has been made for some time, and the pressler type, in which the gassy condition is noted on the press. In a few instances the cheese made from milk inoculated with the culture of *Cl. perfringens* showed little or no gas. The results suggest that some undetermined factor, or factors, played an important part in development of the gassy condition.

Weiser [37] reported that examination of gassy, or split-rind, cheese showed 2 types of organisms, anaerobic bacteria (*Cl. perfringens*) and lactose-fermenting yeasts (*C. pseudotropicalis*). The organisms were conspicuously absent in normal cheese. In extremely gassy cheese both species were present but in mildly defective cheese the latter type was not found. When the farms employed insanitary practices, the fresh milk contained both types.

An organism causing an early-gas defect in cheese was isolated by Tjepkema *et al.*[38] and identified as *Bacillus polymyxa.* After 2.5 months curing at 50 F, a part of the defective cheese was processed at temperatures ranging from 150 to 175 F; the organism could not be isolated from the process cheese.

Abnormal Flavors and Aromas. Swiss cheese sometimes develops abnormal flavors and aromas and these may suggest an objectionable protein decomposition. Factories occasionally have experienced an undesirable fermentation in which the cheese are referred to as "stinkers" because of the foul odor. The defect may be confined to small areas within the cheese so that it cannot be detected from the outside; these areas may be dark in color. Hastings and Frazier [39] found that, in many cases, as the odor increased it was characterized by hydrogen sulfide. Large numbers of butyric acid organisms were found in the spoiled areas, but very few were in the unaffected cheese.

According to Dorner and Thöni,[40] odoriferous fermentation, or gray decay, of cheese appears sometimes after the cheese is made. It is first evident as the *p*H of the cheese rises, and at the end of a few

weeks the cheese has a putrid odor and grayish color. The causative organism is *Bacterium proteolyticum*. When the milk used for the cheese contained as many as 10 of the organisms per ml, secondary odoriferous fermentation ensued. With heavy infection, the cheese shows a high *p*H, a bad odor, gray or dark spots, and gas. Burri [33] reported an objectionable flavor associated with development of a white, crumbly condition and large, irregular holes in parts of the cheese; *Bacillus putrificus* was responsible.

Special Color Defects. Tomka [41] observed round, brown spots (diam about 0.5 mm) on the cut surface or eyes of cheese; frequently, eyes in the cheese were not smooth. In almost all cases the brown spots occurred in cheese with <50 propionic acid bacteria per g. The author concluded that when few propionic organisms were present in a cheese they formed large colonies, which they did not do when many were present. Addition of propionic acid bacteria to the milk for cheese eliminated the brown spots and resulted in better eyes, flavor, and aroma in the cheese.

Under aerobic conditions in whey culture, Virtanen and Kiuru [42] noted that *L. helveticus* formed a brownish-pink color from tyrosine. Colored spots on cheese were located around checking surfaces into which air enters. Accordingly, no foreign bacteria are needed to produce the color defect.

REFERENCES

Swiss Cheese

1. *Zentr. Bakteriol. Parasitenk.*, Abt. 2, **103**, 257 (1941).
2. *J. Dairy Sci.*, **22**, 43 (1939).
3. *J. Dairy Sci.*, **27**, 483 (1944).
4. *Centr. Bakteriol. Parasitenk.*, Abt. 2, **40**, 5 (1914).
5. *J. Dairy Sci.*, **16**, 387 (1933).
6. *J. Dairy Sci.*, **19**, 535 (1936).
7. *J. Bacteriol.*, **27**, 539 (1934).
8. *J. Dairy Sci.*, **18**, 373 (1935).
9. *J. Dairy Sci.*, **18**, 719 (1935).
10. *J. Dairy Sci.*, **36**, 409 (1953).
11. *J. Dairy Sci.*, **24**, 601 (1941).
12. *J. Dairy Sci.*, **24**, 611 (1941).
13. *J. Bacteriol.*, **43**, 181 (1942).
14. *Landw. Jahrb. Schweiz*, **20**, 320 (1906).
15. *J. Bacteriol.*, **6**, 379 (1921).
16. *J. Dairy Sci.*, **6**, 303 (1923).
17. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 499 (1949).
18. *Suomen Kemistilehti*, **B20**, 55 (1947).
19. *J. Dairy Sci.*, **34**, A51 (1951).
20. *Ia. Agr. Expt. Sta. Research Bull.* 264 (1939).
21. *J. Dairy Sci.*, **35**, 915 (1952).
22. *U. S. Dept. Agr. Bur. Animal Ind. Bull.* 151 (1912).
23. *J. Dairy Sci.*, **1**, 91 (1917).
24. *Food Research*, **4**, 81 (1939).
25. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 268 (1949).
26. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 227 (1949).

27. *Intern. Dairy Congr., Proc. 12th Congr.*, **5**, 30 (1949).
28. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 619 (1949).
29. *J. Dairy Sci.*, **12**, 289 (1929).
30. *J. Dairy Sci.*, **35**, 929 (1952).
31. *Milchwissenschaft*, **4**, 97 (1949).
32. *Wis. Agr. Expt. Sta. Bull.* 128 (1905).
33. *Proc. World's Dairy Congr.*, **1**, 278 (1923).
34. *Milchwissenschaft*, **6**, 21 (1951).
35. *Milk Products J.*, **45** (4), 33 (1954).
36. *J. Bacteriol.*, **15**, 203 (1928).
37. *J. Bacteriol.*, **43**, 46 (1942).
38. *J. Dairy Sci.*, **36**, 1272 (1953).
39. *Wis. Agr. Expt. Sta. Bull.* 352, 79 (1923).
40. *Landw. Jahrb. Schweiz*, **51**, 431 (1937).
41. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 623 (1949).
42. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 625 (1949).

BRICK CHEESE

Brick cheese is a semihard, bacteria-ripened variety which, presumably, originated in the United States. Commonly, the body is softer than that of cheddar but not as soft as that of a typical soft cheese. A surface growth of organisms—the smear—develops on the cheese and appears to be essential for production of the typical flavor.

Lactic Acid Streptococci. Culture organisms added to the milk for brick cheese produce lactic acid rapidly during the manufacture. Garey *et al.*[1] found that in a typical trial, in which 0.6% milk culture of *S. lactis* was used in making the cheese, direct counts indicated a gradual increase in numbers of the organism, and there was a small decrease in *p*H between dipping and the sixth hour. Between the sixth and the twelfth hours, the numbers increased rapidly; after the twelfth hour the numbers increased slowly, with the maximum being reached in 3 to 6 days. Cooking to 112 F, in comparison with 106 F, was harmful to later growth and activity of *S. lactis*. *S. thermophilus* never attained appreciable numbers in the cheese. In a typical trial using 0.6% milk culture of *S. thermophilus* in making the cheese, the organism multiplied rapidly in the vat, and after dipping increased steadily until the sixth hour. Between the sixth and the twelfth hours, the numbers increased more slowly and reached their maximum. Cooking to 112 F favored growth and activity of *S. thermophilus*. Late growth of *S. lactis* occurred. When equal proportions of milk cultures of the 2 organisms were used in making the cheese, *S. thermophilus* increased rapidly in the vat and reached its maximum the third hour after dipping. At this time *S. lactis* was just beginning to multiply; it increased rapidly and reached its maximum 12 hr after dipping.

Growth of the culture organisms was slower in cheese from pasteurized milk than in cheese from raw milk.

Rapid production of acid during manufacture of brick cheese is necessary and is dependent on the culture organisms added to the milk. Langhus *et al.*[2] noted that the quality of brick cheese usually is no better than the quality of the culture employed.

Foster *et al.*[3] isolated 1,016 cultures from 18 lots of brick cheese, at different stages of ripening, and found that *S. lactis* was the predominant organism throughout the ripening of cheese in which it was used as part, or all, of the culture. It increased to 1 to 3 billion per g during the first 1 to 4 days and declined after 3 to 4 weeks. It also increased slowly in cheese in which it had not been used as culture and reached several hundred million per g after 1 to 2 weeks. When *S. thermophilus* was used as the culture, it increased rapidly to 1 to 2 billion per g at 12 hr, then decreased rapidly after 1 to 2 weeks and seldom was found later in the ripening.

Using a sweet-curd process for brick cheese, Zorn *et al.*[4] noted that *S. lactis*-type bacteria reached numbers of 1 to 2 billion per g but within 1 or 2 weeks counts of viable organisms dropped to a few million or less. The predominant organisms in the cheese during ripening were micrococci and/or enterococci, but because of their small numbers they appeared to have little to do with the ripening. The authors considered that fine brick cheese can be made in the usual period of 6 to 8 weeks without action of large numbers of viable organisms within the cheese during the ripening.

Other Organisms in Cheese. Lactobacilli have been noted in raw-milk cheese after 1 or 2 weeks but they seldom appeared in pasteurized-milk cheese.[3] *L. casei* was encountered most frequently and in numbers up to 10 to 100 million per g. It was considered the most important organism in bringing about changes in body during the ripening of the cheese and also appeared to contribute to the flavor.

Salting. Byers and Price[5] found that salt penetrated the outer layer of brick cheese quickly, but 8 weeks of curing elapsed before the salt content was practically uniform throughout the cheese. Dry salting was effective but not as uniform as brine salting. Desirable results were obtained when the cheese took up salt to the extent of about 2% of the final weight. Excessive salt caused hard, curdy body, unnatural white color, slow curing, loss of yield, and delayed lactose fermentation, whereas low salt encouraged abnormal fermentations and caused weak body and open texture.

Smear of Cheese. Langhus *et al.*[6] reported that the smear on brick cheese appears only when growth of several types of organisms occurs. Yeast-like organisms develop first and are followed by micrococci and rods of the *Bacterium linens* type. The smear reduces the acidity of the surface layer of the cheese, the decrease being greater when the smear development is luxuriant. The surface sections of brick cheese were the same in water- and alcohol-soluble nitrogenous compounds as the inner sections, which suggests there is no ripening from the outside. However, gradual diffusion of the characteristic surface flavor was obvious. The authors suggest that the slight odor of extensive protein decomposition in brick cheese is produced by the surface organisms and that the characteristic odor is absorbed by the cheese. Curing cheese at 58 to 60 F under conditions that favored activity of the smear organisms gave the cheese more flavor and caused a more rapid protein breakdown, but as measured by analytical procedures the latter occurred uniformly throughout the cheese.

Using contact preparations of the surface of cheese, Iya and Frazier [7] found that cocci developed first, within 3 days. Between 3 and 8 days after salting yeasts appeared, increased in numbers, and gradually disappeared. After about 8 days large numbers of *Bact. linens* were present. The yeast (a *Candida* species) metabolizes the lactates in the cheese and raises the *p*H, which favors growth of *Bact. linens;* also, it supplies some essential growth factor, or factors, for *Bact. linens.*

Bacterium linens. *Bact. linens* is a typical cheese organism in that it grows on the surface of various semihard and soft cheeses and apparently plays a role in the protein breakdown and flavor development. Albert *et al.*[8] noted that in milk it produced an alkaline reaction and then conspicuous proteolysis; amino nitrogen was increased significantly. The organism readily tolerated 15% sodium chloride in broth or skim milk and there was some evidence of growth in considerably higher concentrations. The salt tolerance enables it to grow on the surfaces of various cheeses. It grew at *p*H values of about 6.0 and about 9.8 but not at about 5.0. Corresponding volatile acids were produced from various lower alcohols, for example, acetic acid from ethyl alcohol. Catalase was actively produced. The organism was not lipolytic. *Bact. linens* was found widely distributed in certain dairy products and in materials about stables, especially feed, hay, straw, water, and manure. A special technic, involving use of cheese agar with 5% salt and incubation of the plates in oxygen, facilitates its isolation.

Thomasow [9] found that cell-free culture filtrates prepared from *Bact. linens* were rich in proteases but these enzymes were almost absent from the autolyzed bacterial suspension. Peptidases were present only in small amounts and appeared to be endoenzymes. The casein-splitting enzyme was most active in young cultures and at *p*H 7.0, whereas peptone-splitting activity was slight in young cultures but soon increased (optimum *p*H 6.5). The protease was weakened at 122 F and destroyed at 176 F.

Defects. According to Price and Spicer,[10] the *p*H of cheese 3 days after making is a useful index of the acid characteristics of the ripened cheese; it should not be below 5.1.

Garey *et al.*[1] found that when *S. lactis* alone was used as the culture, control of the moisture at dipping was necessary to avoid sour flavor and weak or crumbly body; if the moisture content at 2 days was above 42%, the defects appeared. With *S. thermophilus* alone as the culture, fermented flavor and open texture developed in the cheese. A combination of *S. lactis* and *S. thermophilus* as the culture gave a better cheese than either organism alone. Foster *et al.*[11] reported that when the moisture content of the cheese remained above 39%, fermentation of all the lactose lowered the *p*H to about 4.8 and acid flavor and short crumbly body resulted. In an attempt to prevent development of excessive acidity, they used a washing procedure to remove some of the lactose from the curd before dipping. After this treatment enough fermentable lactose remained in the curd to lower the *p*H to about 5.00 to 5.15 in cheese with a moisture content of 40 to 42%. A mild wash, in which 25 to 28 lb of water per 100 lb of milk was added to the curd plus whey in the vat and then 50 to 55 lb of whey was removed and replaced by an equal volume of water at 90 F, gave the best results, the cheese having a mild clean flavor, soft smooth body, and medium close texture. Undesirable fermentations were more common in washed curd than in conventional cheese, presumably because of the lower acidity in the former. With good-quality milk and a mixed culture (*S. lactis* and *S. thermophilus*), undesirable fermentations did not appear and the washed curd cheese was superior in flavor, body, and moisture content.

Hanson *et al.*[12] found that lack of acid development, low salt content, and large loaves of cheese tend to encourage development of late gas in brick cheese but that these are not necessarily the only factors involved. Late gas formation by anaerobic spore-forming bacteria has been noted [3] whenever the *p*H did not drop below 5.3 during the first 3 days of ripening. The defect was most pronounced in cheese made

with *S. thermophilus* from pasteurized milk by the washed curd method.

Bitterness in brick cheese has been attributed to excessive numbers of *S. liquefaciens.*[3]

REFERENCES

Brick Cheese

1. *J. Dairy Sci.*, **24**, 1015 (1941).
2. *J. Dairy Sci.*, **22**, 101 (1939).
3. *J. Dairy Sci.*, **25**, 323 (1942).
4. *J. Dairy Sci.*, **35**, 1067 (1952).
5. *J. Dairy Sci.*, **20**, 307 (1937).
6. *J. Dairy Sci.*, **28**, 827 (1945).
7. *J. Dairy Sci.*, **32**, 475 (1949).
8. *Ia. Agr. Expt. Sta. Research Bull.* 328 (1944).
9. *Kiel. Milchwirtsch. Forsch. Berichte*, **2**, 35 (1950).
10. *J. Dairy Sci.*, **21**, 615 (1938).
11. *J. Dairy Sci.*, **25**, 313 (1942).
12. *J. Dairy Sci.*, **21**, 433 (1938).

BLUE-VEINED CHEESES

The blue-veined cheeses are semihard, mold-ripened varieties in which the characteristic mold, *Penicillium roqueforti,* produces blue or bluish green veins during curing. The mold is active in bringing about changes in flavor and texture. Representatives of this group of cheeses are the French roquefort which is made from sheep's milk and the Italian gorgonzola, which is made from cows' milk; various countries, including the United States, produce blue cheese from cows' milk.

Milk Used. Commonly, blue cheese is made from raw milk because fat hydrolysis is an important part of the ripening and milk lipase aids in it. By homogenizing the milk, Lane and Hammer [1] greatly accelerated the ripening of blue cheese, much of which is now made from homogenized milk or a mixture of skim milk and homogenized cream. With poor-quality milk, bacterial defects may appear in raw-milk cheese; these can be controlled partly by pasteurization of the milk, but then ripening is delayed because, with the milk lipase destroyed, all the fat hydrolysis must be brought about by the mold. Peters and Nelson [2] found that cell-free lipase from cultures of *Candida lipolytica* was useful in ripening blue cheese from pasteurized, homogenized milk. Nutrient broth [3] containing 0.05% glucose and special buffer was most favorable for lipase production by *C. lipolytica.* Accumulation of lipase in the medium was favored by growing the culture without shaking or aeration, but added reducing substances had little or no effect. Lipase activity [4] on butterfat was demonstrated

over a *p*H range of 4.0 to 8.0, 6.2 to 6.5 being optimum. Salting out of lipase with saturated ammonium sulfate plus 30% ethyl alcohol resulted in a white precipitate high in lipase activity.

Often the fat in milk for blue cheese is bleached so that the cheese will have a light color. This commonly is accomplished by separating the fat as cream and adding a reagent, such as benzoyl peroxide, under proper temperature conditions.

S. *lactis* in Blue Cheese. In making blue cheese, acid development occurs through activity of *S. lactis;* its presence in the milk is assured by addition of lactic culture. If acid does not develop properly, the cheese fails to drain normally and growth of objectionable bacteria may occur. After reaching large numbers, *S. lactis* soon dies. Evans [5] found that it disappeared from cheese in about 2 or 3 weeks and ascribed this to the high concentration of salt. However, products of fat hydrolysis in the cheese may be a factor.

In trials by Coulter *et al.*,[6] blue cheese developed a *p*H of about 4.7 within 24 hr of manufacture. Except for a temporary drop following piercing, the *p*H gradually increased to about 6.5 after about 3 months and then gradually decreased to about 5.7 after 9 months.

In some instances the *p*H of blue cheese reaches relatively high values, presumably when the mold develops extensively, and such values often are accompanied by darkening of the cheese and undesirable flavors.

Bacteria Other than *S. lactis*. Various bacteria, such as lactobacilli, liquefiers, streptococci other than *S. lactis,* etc., have been found in blue cheese but apparently play no part in the ripening. They are present because of their presence in the milk and their ability to survive conditions in the cheese.

Mold of Blue Cheese. The typical mold of blue cheese was named *P. roqueforti* by Thom.[7] In some of the older literature it is referred to as *Penicillium glaucum*.[8] *P. roqueforti* is not limited to blue cheese, and frequently is found in other dairy products. It has been isolated from silage and, undoubtedly, is more or less regularly present in the stable surroundings.

In making blue cheese, mold spores, in the form of a powder, are mixed with the curd just before it is put into the hoops or are sprinkled on the curd in the hoops. The powder commonly is prepared by growing the mold on bread, or other material, and then drying and grinding. Hussong and Hammer [9] sterilized small cubes of whole wheat bread in wide-mouth bottles and then inoculated and incubated them. After a heavy growth was obtained, the bread was dried and

ground. Powders prepared in this way contained 30 million to 1.5 billion spores per g, as determined by plating. When the spores were suspended in water and counted directly, the values were much higher; many of the spores were in groups.

After the cheese has drained properly, it is salted, by either brine or dry salting, to a relatively high salt content and then pierced to admit air to the interior of the cheese. Both these procedures have a definite effect on mold growth in the cheese.

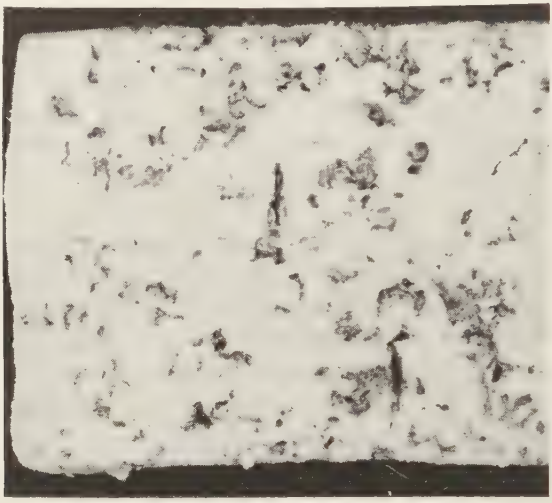


Figure 47. Mold growth in blue cheese.

The dominance of *P. roqueforti* in blue-veined cheeses, when spores and vegetative growth of various molds may be present as a result of contamination during production, presents a problem in the relationship of the organism to the environment. Thom and Currie [10] analyzed the gas in both imported and experimental cheese and found the percentage of free oxygen low, 7.0% being the highest value in 5 analyses. The carbon dioxide varied from 21.1 to 40.9%; the percentages appeared to be highest in comparatively young cheese and to fall off through diffusion as the cheese aged. The investigators studied a number of species of *Penicillium* and *Aspergillus* and noted that *P. roqueforti* tolerated a high carbon dioxide content and low oxygen content better than any of them. However, *G. candidum* also grows at a low oxygen tension. Thom *et al.*[11] found this species would develop in cheese when the salt concentration was 2%, but all abnormali-

ties due to it disappeared with 4% salt in the newly made cheese. Accordingly, it appears that mold growth in cheese is limited largely to *P. roqueforti* because it tolerates both low oxygen tension and high salt concentration and other molds do not.

Certain lots of blue cheese show areas of mold growth that definitely are not *P. roqueforti*. In many cases these represent invasion from the surface of the cheese but, occasionally, they appear to originate in the interior. Irregularities in composition of the cheese may be responsible for most of the areas, but some of them appear to develop in cheese that is quite normal.

Knight *et al.*[12] obtained strains of *P. roqueforti* with white spores by ultraviolet-induced mutation of the usual mold. The white mutants showed no marked changes in lipolytic, proteolytic, or growth rates. Normal *P. roqueforti* seemed to require more iron for good growth than may be present in mixed milk, and the mutants required less iron than the parents. Morris *et al.*[13] reported that excellent cheese can be made with a white mutant of *P. roqueforti;* its color, body, texture, and mild flavor are different enough from blue cheese to constitute a new type.

Proteolytic Action of *P. roqueforti*. The proteolytic action of *P. roqueforti* is evident from the appearance of milk cultures. When a cheese is not pierced, failure of the mold to grow, due to lack of air, quickly is reflected in the body of the cheese. Instead of the curd breaking down normally, it remains tough and crumbly. This suggests that *P. roqueforti* is the principal proteolytic agent in blue cheese. Currie [14] reported data on the nitrogen distribution in ripened roquefort cheese; 45.5% of the nitrogen was in insoluble forms, 10.7% as caseoses, 8.6% as peptones, 29.1% as amino acids, and 6.1% as ammonia.

Thomasow [15] found the following products resulted from protein decomposition in fully-ripened blue mold cheese: Volatile basic compounds (ammonia and amines); nonvolatile basic compounds (histidine, arginine, etc.); volatile acids (caproic, valeric, and butyric); nonvolatile acid (probably succinic); and traces of aldehydes.

Thibodeau and Macy [16] reported that on a 1% casein substrate with phosphate buffer the protease of *P. roqueforti* reached its optimum activity between *p*H 5.8 and 6.3. The mold seemed to produce only one protease which appeared to be of the trypsin type but differed from animal trypsin in that its optimum activity occurred at a lower *p*H and over a wider *p*H range. Concentrations of sodium chloride such as exist in blue cheese did not retard action of the protease.

Lipolytic Action of *P. roqueforti*. Fat hydrolysis during ripening of cheese was studied in detail by Currie,[14] who pointed out that it is due chiefly to a water-soluble lipase produced by the mold. The hydrolysis eventually results in the accumulation, in free and combined forms, of the acids present in milkfat; of these, caproic, caprylic, and capric acids are especially important. With growth of the mold on media to which milkfat had been added, it appeared that more than half the fat had been hydrolyzed. Solubility of the lipase in water is of special interest in that it explains fat hydrolysis at points in the cheese which are free of mold.

Foder and Chari [17] found a *p*H optimum of 6.5 for mycelial extracts of *P. roqueforti* whereas that of the lipase secreted into a growth medium was 8.0. Morris and Jezeski [18] also studied lipolytic activities of preparations from mycelium of *P. roqueforti* and from a growth medium of the organism. The temperature optima on tributyrin and milkfat were between 86 and 89.6 F with 1 hr holding. The *p*H optima were as follows: Mycelial preparation 6.0 to 6.7 on tributyrin and 7.0 to 7.2 on milkfat; medium preparation 7.0 to 7.8 on tributyrin and 6.5 to 6.8 on milkfat. The lipase system was one-third as active on milkfat as on tributyrin. Activity of the system on both triglycerides and ethyl esters decreased as the molecular weight of the esterified fatty acids increased; it also was decreased by an increase in sodium chloride. Differences in the 2 lipase preparations suggest that there may be more than one lipase present in the mold.

When *P. roqueforti* was grown in Czapek's solution with milkfat added by Imamura and Tsugo,[19] there was an accumulation of volatile fatty acids, but, when sugar was omitted from the medium and fat was the only source of carbon, the mold utilized fatty acids and there was little accumulation of them. The fat hydrolyzing activity of an enzyme extract of the mold was highest in the *p*H range of 5 to 6. Low molecular weight fatty acids were decomposed by *P. roqueforti* but not higher acids, which explains the accumulation of insoluble volatile acids in cheese.

Action of *P. roqueforti* on Acids. *P. roqueforti* is favored in its growth by organic acids that, presumably, supply a suitable carbon source. Lactic acid is of importance in this connection. Some of the acids liberated from milkfat also are used by the mold. Bryant [20] noted that when volatile fatty acids were added to milk inoculated with *P. roqueforti* there was destruction of the acids. Reduction was largest with butyric acid and became less as the molecular weight increased. It is probable that during the early stages of the ripening

of cheese the lower fatty acids liberated are used by the mold, and that when conditions become less favorable for the mold the acids tend to accumulate.

Hammer and Bryant [21] found that cultures of *P. roqueforti* in milk containing caprylic acid developed an odor suggestive of blue cheese. The compound responsible was identified as methyl-*n*-amyl ketone. It appears to be formed from caprylic acid, through action of *P. roqueforti* under unfavorable growth conditions, as a result of a beta oxidation and elimination of carbon dioxide from the carboxyl group. Heitzman [22] noted that nonproliferating mycelium brought about the same type of change in caproic acid with formation of methylpropyl ketone. Stokoe [23] concluded that various methyl ketones may be formed by molds because absorption of poisonous fatty acids by the mycelium impedes respiration.

Characteristic Flavor of Blue Cheese. Currie [14] pointed out that the flavor of cheese is due largely to the free and combined forms of caproic, caprylic, and capric acids liberated from the milkfat. These compounds have a sharp, peppery taste and are responsible for the characteristic effect when the cheese is eaten. Since fats of the milk of various species of animals differ in composition, a difference in suitability of milk from the various species for the manufacture of blue cheese might be expected. Currie investigated this from the standpoint of the use of cows' milk and concluded that differences between the fats from typical roquefort cheese and cows' milk are not great enough to necessitate exclusive use of sheep's milk in the manufacture of this type of cheese; however, cheese made from sheep's milk may be expected to have more of a peppery taste.

The odor of methyl-*n*-amyl ketone is rather definite in many lots of cheese and appears to be a significant flavor contributant. In some lots, it is not evident although it may be present to some extent; occasionally, it is so pronounced it is objectionable. Stärkle [24] distilled roquefort cheese and obtained about 2 drops of material that had an intensive odor of methyl-*n*-amyl and methyl-*n*-heptyl ketones. The amount was too small to permit separation and identification. Methyl-*n*-heptyl ketone apparently is formed from capric acid. Patton [25] recovered material from blue cheese that contained a high concentration of methyl ketones. Distillation yielded relatively pure fractions of methyl-*n*-propyl, methyl-*n*-amyl, and methyl-*n*-heptyl ketones. Sjostrom and Malm [26] found that, in culture media, *P. roqueforti* consumed the fatty acids up to and including myristic with production of

"roquefort" aroma from caprylic acid and to a lesser extent from caproic and butyric acids.

Slime on Cheese. Early studies indicated that slime formation on cheese is not essential to ripening. More recently, Morris *et al.*[27] found that initially the surface microflora of blue cheese consists primarily of yeasts and some mold; later, cocci and rods predominate. The slime becomes alkaline during the ripening and the acidity of the cheese is reduced. There is accelerated ripening in portions of cheese taken 0.125 in. from the surface. Exposing the cheese to excessive or prolonged slime formation is detrimental to the flavor and color of the cheese and to the color of the mold in the cheese.

Hartley and Jezeski[28] also noted that the initial flora of the slime on blue cheese was predominately yeasts and some mold (*P. roqueforti*) and was followed by an increase in cocci and rods. The organism predominating in the slime of cheese ripened at 46 to 49 F was *Bacterium erythrogenes*. *Bact. linens* was the dominant rod in the slime of cheese cured at 55 to 58 F but was absent with curing at 46 to 49 F.

Normal slime formation often is an index to satisfactory humidity in the curing room. Its removal by washing or scraping before wrapping of the cheese, and perhaps also during curing, involves labor and loss in weight of the cheese so that certain manufacturers have attempted to limit slime formation by partially covering the surface with parchment or waxed paper or by dipping the cheese in wax before piercing.

Species of Molds in Blue-veined Cheeses. It often has been assumed that *P. roqueforti* is the mold regularly associated with all blue-veined cheeses. However, Steuart[29] found that, in 12 such cheeses of different varieties, this organism was thoroughly dominant in 8, was present in another, and apparently was absent in 3. Lane[30] compared 8 strains of penicillia from the standpoint of flavor and volatile acid produced in blue cheese. Certain strains regularly were associated with cheese lacking in flavor. There was a definite difference in quality of the cheese produced with the strains identified as *P. roqueforti*.

It is probable that various mold species can bring about the general changes necessary in the ripening of cheese, although *P. roqueforti* has been most useful for this purpose. Satisfactory blue cheese has been made with *Penicillium oxalicum*.

Factors Influencing Growth of *P. roqueforti*. The great differences in mold growth in cheese suggest there are various influencing factors.

Golding [31] noted that acetic and citric acids had an effect on the growth of different strains of *P. roqueforti.* Low concentrations of acetic acid tended to reduce digestion of casein in milk, while citric acid tended to increase it. Studies on salt tolerance of *P. roqueforti* [32] suggested that the salt concentrations usually found in cheese may retard but not inhibit growth of the mold. Blue cheese was subjected to high pressures in an iron cylinder during ripening,[33] using both continuous and intermittent treatments. The procedures accelerated mold growth somewhat and the intermittent method gave the better results.

Golding [34] concluded that it is the presence of carbon dioxide, rather than absence of oxygen, that is a limiting factor in the growth of *P. roqueforti* in cheese and that piercing the cheese aids mold growth by permitting escape of carbon dioxide. Three strains of *P. roqueforti* were grown at 7 temperatures in atmospheres from 21 to 2.1% oxygen, obtained by adding nitrogen to air.[35] Only with the greatest dilution of oxygen (2.1%) was there a significant reduction in growth. It appeared there is a tendency for a shortage of oxygen to reduce growth more at higher temperatures. Relatively small concentrations of carbon dioxide in air increased the growth of *P. roqueforti,* and larger concentrations inhibited it; [36] acceleration or inhibition was apparent sooner at lower temperatures.

Factors Influencing Ripening. The finely divided fat obtained by homogenizing milk for blue cheese hydrolyzes more readily than fat in the usual condition so that changes in the fat progress rapidly. With homogenized milk, fat hydrolysis due to action of milk lipase often is evident before the curd is in the hoops. Homogenization also results in a soft body, which permits spreading of the cheese, and in comparatively white color.

Coulter *et al.*[37] noted that, within the range of values used, variations in acidity during manufacture of blue cheese did not appear to be highly important. Cheese set and dipped at low acidities ripened somewhat sooner than those set and dipped at high acidities, but excellent cheese was produced with all acidities used.

According to Coulter and Combs,[38] addition of steapsin to the milk or curd accelerated the ripening of cheese. With commercial steapsin added, approximately the same flavor development was obtained in 5 months as the control cheese showed in 12 months. Steapsin gave the cheese a bitter flavor. Cheese made with steapsin, but without mold, became rancid but did not develop the typical blue cheese flavor.

Lane and Hammer [39] found that addition of 2% salt to blue cheese curd just before hooping sometimes resulted in better mold growth in the cheese, rather regularly gave a lighter color, and tended to control certain flavor defects. The better mold growth probably was due to the more open texture resulting from the salting, the lighter color to an increase in stability of the curd that kept the fat dispersed, and the tendency to control flavor defects to action of salt on certain undesirable organisms. Some of the salt added to the curd drains away in the whey but no serious irregularities occurred in the salt content of the finished cheese.

Figure 48. Blue cheese curing cave. Courtesy F. M. Frederiksen.

Thibodeau and Macy [16] reported that addition to blue cheese at hooping of the enzymes of *P. roqueforti* in the form of mycelium (6 g per 5-lb cheese) produced cheese of fine quality in one-half the normal ripening period.

Defects of Blue Cheese. Because *P. roqueforti* is the primary ripening agent in blue cheese, its proper development is important. If it does not develop sufficiently, the cheese lacks blue color and flavor and is too firm. In some cases the mold in an uncut cheese loses its color but regains it quickly when exposed to air. Excessive mold growth in blue cheese often results in a musty, unclean flavor and the cheese lacks the flavor of methyl-*n*-amyl ketone.

Proper mold development in cheese is dependent on proper texture in the cheese and adequate control of the ripening conditions. The strain of mold used also is of extreme importance. An outbreak of poor mold growth apparently was due to use of an atypical strain of *P. roqueforti* in preparing the mold powder.[40] Funder [41] noted that morphologically similar mold cultures sometimes behave quite dif-

ferently with regard to decomposition of milk, even strains of the same species being quite different in enzymes, and suggested that commercial cultures of *P. roqueforti* should be examined closely before being used in cheese. Particular attention should be given to formation of mycelium and reaction produced in cheese.

Sjostrom and Malm [26] reported that when blue cheese was manufactured by a process which resulted in rapid acid formation (pH <5.1 in 6 hr) the cheese developed cracks.

Gas holes occasionally are noted in commercial cheese but the defect is less common than might be expected since blue cheese so often is made from raw milk. The open texture of blue cheese may be a factor. Bryant and Hammer [40] reported that trials with a culture of *A. aerogenes* recently isolated from gassy cheddar cheese showed that inoculations into milk which resulted in very gassy cheddar cheese caused no significant gas holes in blue cheese; the blue cheese was made from homogenized milk and early production of fatty acids may have inhibited the gas former.

Cheese occasionally develops a defect in which a portion or all of the edges of a cheese become soft.[40] There is no gradual softening as the defective edge is approached and the dividing line between firm and soft cheese is sharply defined. The surface of the soft edge readily is slipped off, exposing soft cheese beneath that has the color and consistency of camembert; with age, the odor of the soft cheese suggests limburger. The soft edge has a higher moisture and lower salt content than the normal cheese and also a higher pH and higher bacterial content. The defect has been produced by placing normal cheese near a humidifier where free moisture could strike it. Various conditions in curing rooms, such as occasional excessive cooling of cheese near brine coils, can result in accumulation of moisture on cheese. Softening would be expected to occur most readily at an edge since the two surfaces are separated by a relatively small amount of cheese and the ratio of deposited moisture to cheese is high.

In cheese that has ripened for some time, gray discoloration is a common defect.[40] It is most commonly noted at the surface, especially when the slime is removed, and tends to spread through the entire cheese. Extensive discoloration usually is accompanied by a mousy, ammoniacal flavor that becomes soapy with age. The defect seems to be confined to certain lots of cheese but may not involve all the cheese in a lot. It does not spread from one cheese to another. The defect is accompanied by an increase in pH. The variation from the normal ripening mechanism that causes the defect presumably in-

volves formation of basic products from protein. Contaminants capable of reproducing the defect have not been isolated.

A black discoloration and musty flavor in cheese have been attributed to growth of *Hormodendrum olivaceum*, particularly in pierce holes and surface cracks.[10] Under experimental conditions growth of the mold was confined to the outside of the cheese and to pierce holes, but it appeared that with cheese having a body susceptible to cracking the mold could cause extensive damage.

In certain curing rooms red areas of mold growth often appear on cheese. Commonly, the mold is first evident as small bright-red colonies that may be few or numerous. The colonies usually are evident in 10 days to several weeks and increase in size until they are 1 in. or more in diameter. They may grow together and form large red areas which occasionally make up a considerable portion of the cheese surface. Hammer and Gilman [12] easily isolated the mold on a special cheese agar but not on various other media. They considered the designation should be *Sporendonema casei*, although various other names have been employed. Covering the cheese, for example, with wax or with petrolatum containing mold inhibitor, aids in controlling growth of the mold when normal cleaning of the cheese does not.

REFERENCES

Blue-Veined Cheeses

1. *Ia. State Coll. J. Sci.*, **10**, 391 (1936).
2. *J. Dairy Sci.*, **31**, 611 (1948).
3. *J. Bacteriol.*, **55**, 581 (1948).
4. *J. Bacteriol.*, **55**, 593 (1948).
5. *J. Agr. Research*, **13**, 225 (1918).
6. *J. Dairy Sci.*, **21**, 273 (1938).
7. *U. S. Dept. Agr., Bur. Dairy Ind. Bull.* 82 (1906).
8. *U. S. Dept. Agr., Bur. Dairy Ind. Bull.* 118 (1910).
9. *J. Dairy Sci.*, **18**, 599 (1935).
10. *J. Biol. Chem.*, **15**, 249 (1913).
11. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 79 (1914).
12. *J. Dairy Sci.*, 33, 929 (1950).
13. *J. Dairy Sci.*, **37**, 711 (1954).
14. *J. Agr. Research*, **2**, 1 (1914).
15. *Milchwissenschaft*, **2**, 354 (1947).
16. *Minn. Agr. Expt. Sta. Tech. Bull.* 152 (1942).
17. *Enzymologia*, **13**, 258 (1949).
18. *J. Dairy Sci.*, **36**, 1285 (1953).
19. *Intern. Dairy Congr., Proc. 13th Congr.*, **4**, 622 (1953).
20. *B. W. Hammer Panegyric*, 119, 1937. Collegiate Press, Inc., Ames, Ia.
21. *Ia. State Coll. J. Sci.*, **11**, 281 (1937).
22. *Compt. rend.*, **218**, 859 (1944).
23. *Biochem. J.*, **22**, 80 (1928).
24. *Biochem. Z.*, **151**, 371 (1924).
25. *J. Dairy Sci.*, **33**, 680 (1950).
26. *Svenska Mejeritidn.*, **44**, 81 (1952).
27. *J. Dairy Sci.*, **34**, 209 (1951).
28. *J. Dairy Sci.*, **37**, 436 (1954).
29. *J. Dairy Sci.*, **2**, 407 (1919).

30. *B. W. Hammer Panegyric,* 107, 1937. Collegiate Press, Inc., Ames, Ia.
31. *J. Dairy Sci.,* **9,** 28 (1926).
32. *J. Dairy Sci.,* **9,** 236 (1926).
33. *B. W. Hammer Panegyric,* 113, 1937. Collegiate Press, Inc., Ames, Ia.
34. *J. Dairy Sci.,* **20,** 319 (1937).
35. *J. Dairy Sci.,* **23,** 879 (1940).
36. *J. Dairy Sci.,* **23,** 891 (1940).
37. *J. Dairy Sci.,* **21,** 239 (1938).
38. *J. Dairy Sci.,* **22,** 521 (1939).
39. *J. Dairy Sci.,* **23,** 169 (1940).
40. *Ia. Agr. Expt. Sta. Research Bull.* 283 (1940).
41. *Intern. Dairy Congr., Proc. 12th Congr.,* **2,** 463 (1949).
42. *J. Dairy Sci.,* **27,** 413 (1944).

LIMBURGER CHEESE

Limburger cheese is a soft, bacteria-ripened variety that was first made in Belgium. It is characterized by a surface smear that plays an important role in the ripening.

Lactic Acid Streptococci. There is a rapid drop in *p*H of limburger cheese after manufacture because of activity of the lactic acid streptococci present in the milk used or added as culture; this activity is favored by the relatively high moisture content of the cheese and by the holding temperature. Yale [1] found the *p*H drop was most rapid during the 24 hr following dipping. At the end of that period the *p*H values of cheese made from raw milk without culture ranged from 4.72 to 5.04 in July and August and from 5.08 to 5.10 in October. At 7 days, values for the July and August cheese were slightly higher than at 24 hr and, with one exception when there was a drop in *p*H, ranged from 5.05 to 5.23. As the cheese aged, the *p*H values increased and ranged from 6.53 to 6.98 at 70 days. When the cheese were fully ripe (70 days), average *p*H values of July and August cheese and of October cheese were about the same, being 6.79 and 6.64, respectively. Undoubtedly, the curing temperature is a factor in the *p*H changes. Pasteurization retarded but did not stop acid development. At 24 hr, cheese made without culture from (1) raw milk, (2) milk heated to 145 F without holding, (3) milk held 15 min at 145 F, and (4) milk held 30 min at 145 F had *p*H values of 4.72, 4.85, 4.95, and 5.05, respectively. At 7 days, the values were about the same for raw- and pasteurized-milk cheese. Addition of 0.05 to 0.2% lactic culture to partly or completely pasteurized milk hastened acid development so that at 24 hr the *p*H values usually were about 5.0, being appreciably lower than without culture. During ripening, *p*H values of cheese from milk containing culture tended to increase more slowly than the values of raw- or pasteurized-milk cheese made without culture. In

one series, *p*H values at 70 days were 6.90 and 6.95 for cheese made without culture, compared to 6.16 and 5.89 for cheese made with culture.

Kelly and Marquardt [2] noted that the *p*H was not uniform throughout a cheese. In general, the *p*H was higher just beneath the surface than at the center. As soon as a definite smear developed on the cheese, the *p*H of the smear was higher than that of the cheese itself, due to activity of the organisms there.

Smear of Cheese. Development of a normal smear is an important factor in the ripening of cheese. Kelly [3] found that the microbiological changes on the surface of ripening cheese showed a very definite sequence. Budding yeasts appeared in 2 to 3 days and were in large masses in 4 to 5 days. At this stage the cheese surface was slimy and the organisms could be distributed evenly over it by rubbing. In 6 or 7 days, *Bact. linens* appeared and by about the eighth day it had increased greatly and was evenly distributed over the whole surface; apparently, this organism was responsible for the reddish surface color that developed. In 10 to 18 days, the yeast cells tended to become smaller and distorted and then disappeared entirely. Very few yeasts cells were found on older cheese. Other organisms sometimes were present in the smear but did not appear to be important in the ripening. The predominant yeast formed a pellicle on liquid media, tolerated salt concentrations as high as 18 to 20%, and did not produce spores.

Yale [4] determined at 2 factories the numbers of organisms in the smears of limburger cheese from 2 to 14 days old. The maximum yeast counts of 900,000,000 and 6,300,000,000 per g were found on the fourth day; both counts were followed by decreases. The maximum bacterial counts of 62,000,000,000 and 360,000,000,000 per g were found on the tenth and fourteenth days, which were the last examinations. Many of the bacteria in the smear were salt-tolerant and grew on a medium containing 7.5% salt. The predominant group of bacteria in the smear was strictly aerobic, gram-positive, nonchromogenic, nonspore-forming rods that produced a marked alkaline reaction in litmus milk in 1 month at 77 F and did not liquefy gelatin. *Bact. linens* was next in importance. Inoculation of the surface of raw-milk limburger cheese with *Bact. linens* failed to improve the quality of the cheese over that naturally inoculated under good plant conditions, but addition of milk cultures to pasteurized milk improved the flavor in the majority of the lots of cheese made in 4 trials at 2 factories.

The conditions that largely determine the flora of the smear include

salt concentration and *p*H. Kelly and Marquardt [2] outlined the sequence of changes involved. Soon after salting, the salt concentration is highest in the surface layer of the cheese and low in the interior; the gradual penetration results in an equilibrium in about 10 days. Organisms on the surface of young cheese are in a high salt concentration and this excludes many types while the low *p*H excludes still others. The yeasts on the cheese tolerate both conditions and act on lactate and protein to raise the *p*H above the point (*p*H 5.85) at which *Bact. linens* can grow. The high salt concentration still prevents growth of many species. *Bact. linens* raises the *p*H still higher through its action on proteins and is believed to complete the ripening. The aerobic nature of *Bact. linens* and its inability to grow below *p*H 5.85 presumably prevent its development in the interior of the cheese; this suggests that the buttery texture and some of the flavor are produced by enzymes of *Bact. linens* rather than by the organism itself. Purko *et al.*[5] reported data indicating that the yeasts on limburger cheese contribute to the growth of *Bact. linens* by decreasing the acidity of the curd and by simultaneously secreting required growth factors.

Apparently, under the usual conditions the cheese are inoculated with the needed surface organisms from equipment, shelving, etc., in the plant. In addition, the organisms probably are present, at least in small numbers, in the milk coming from the farms.

Pasteurization of Milk. Yale [1] found that either partial or complete pasteurization markedly improved the quality of cheese made in July and August from poor-quality milk but not that made in October from better-quality milk. Heating 30 min at 145 F resulted in as good cheese as partial pasteurization, using holding periods of 0, 10, or 20 min. Cheese from pasteurized milk had a milder limburger flavor than that from raw milk. Flavor development in the former varied with the different lots and usually was most pronounced without added culture. With some lots, culture was not beneficial, whereas with others addition of 0.05 to 0.1% improved the grade of the cheese. Pasteurization of milk with proper manufacturing methods resulted in a product of uniform quality.

Gas Production by Cheese in Tins. Dorn and Dahlberg [6] found that the volume of gas produced by limburger cheese in tins was greatly increased by allowing the cheese to ripen for 2 weeks under aerobic conditions before canning. This preliminary ripening gave cheese of good quality, including a smooth body. With canning immediately after salting, the curd remained acid and never ripened.

Vacuum packing, compared with sealing at atmospheric pressure, had but slight effect on rate of gas production or types of gases produced; the total volume of evolved gas tended to be slightly lower with vacuum packing. With fresh limburger curd, carbon dioxide constituted up to 95 to 97% of the gases given off and hydrogen 3 to 4%. With partially ripened curd, the volume of gases was 4 to 5 times as great as with fresh curd; carbon dioxide made up 90 to 95% and hydrogen 5 to 10%. The gases evolved per pound of cheese canned when partially ripe were 2 to 3 liters during 18 weeks at 50 F and 1.5 liters at 40 F; they represented a loss of 0.6 to 1.2% of the weight of the cheese. The steam distillate of the cheese contained ammonia and indol. Hydrogen sulfide was present in the gases evolved. The volatile fatty acids of the cheese included acetic and propionic but were chiefly higher fatty acids, probably resulting from fat hydrolysis.

Defects. Investigations of Kelly [7] showed that gas in limburger cheese was due to growth of *E. coli* and *A. aerogenes*. The relatively high temperatures maintained during the making and the low acidity of the milk encouraged growth of these organisms so that gas was produced in the cheese when as few as 12 per ml were present in the milk. More gas was produced when the proportion of coliform organisms to other organisms was high than when it was low. Good cheese was made from milk heated sufficiently to control gas producers.

Kelly [3] occasionally encountered molds on limburger cheese and, whenever the numbers were significant, the cheese showed the wrinkled appearance that usually occurs with extensive development of *G. candidum*. Kelly and Marquardt [2] studied 2 lots of cheese in which the drop in *p*H did not go below 6.0 and was followed by a steady rise; the cheese developed a strong putrid odor. The results indicate acid development is necessary to control putrefactive bacteria. Use of large amounts of culture in heated milk [1] has given cheese which developed a body, texture, and flavor more like brick than limburger cheese.

REFERENCES

Limburger Cheese

1. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 253 (1940).
2. *J. Dairy Sci.*, **22**, 309 (1939).
3. *J. Dairy Sci.*, **20**, 239 (1937).
4. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 268 (1943).
5. *J. Dairy Sci.*, **34**, 699 (1951).
6. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 266 (1942).
7. *J. Dairy Sci.*, **22**, 299 (1939).

CAMEMBERT CHEESE

Camembert cheese is a soft, mold-ripened type that originated in France. The characteristic mold, *Penicillium camemberti*, and also bacteria and yeasts, grow at the surface and bring about the ripening through elaboration of enzymes that diffuse toward the center of the cheese. Since ripening follows these enzymes rather closely, the outer portion of the cheese ripens first; often there is a sharp differentiation between the creamy, waxy, ripened cheese and the unripened curd.

Organisms in Cheese. Acid formation in cheese is brought about mainly by lactic acid streptococci. Their abundance in the fresh curd is assured through use of lactic culture in the milk. They encounter conditions favorable for growth because of the retention of considerable quantities of water and materials in solution and develop until large numbers are present. Esten and Mason [1] found that the highest counts usually occurred about the third day when there were up to several billion organisms per g. A decrease then began and continued, with fluctuations, until the cheese was ripe. Usually 99% or more of the bacteria in the interior of the cheese were of the lactic acid type.

Other organisms also are present in cheese more or less constantly. They include liquefying bacteria, lactobacilli, and yeasts. In general, these organisms are present because of their ability to develop at a low *p*H. They apparently are not important in the ripening.

Molds of Camembert Cheese. *P. camemberti* generally is regarded as the typical mold of camembert cheese; a white variety is designated *P. camemberti* var. *rogeri*. The species seems to be limited in its growth mainly to camembert cheese and is not widely distributed.

Early studies on cheese suggested that, for normal ripening, growth of both *P. camemberti* and *G. candidum* on the surface is required. According to this idea, if *G. candidum* is absent or limited in its growth, ripening may appear to progress normally but the cheese lacks flavor, whereas, if overdevelopment occurs, surface growth on the cheese is thick and wrinkled, it does not adhere properly, and *P. camemberti* fails to develop as it should. Nutrients and moisture available at the surface of the cheese are satisfactory for *G. candidum*, but the salt present tends to restrain it.

A later idea is that growth of *G. candidum* is not necessary in the ripening of cheese, and the product has been made with extreme precautions to limit growth on the cheese surface of all organisms except *P. camemberti;* these precautions include pasteurization of

the milk, thorough steaming of all the equipment with which the milk or cheese comes in contact, heavy inoculation of the young cheese with *P. camemberti,* and great care in the curing rooms. Some of the cheese made on this basis has been satisfactory for many consumers, but various defects have been encountered in certain lots.

Another recent idea is that *G. candidum* is the important organism

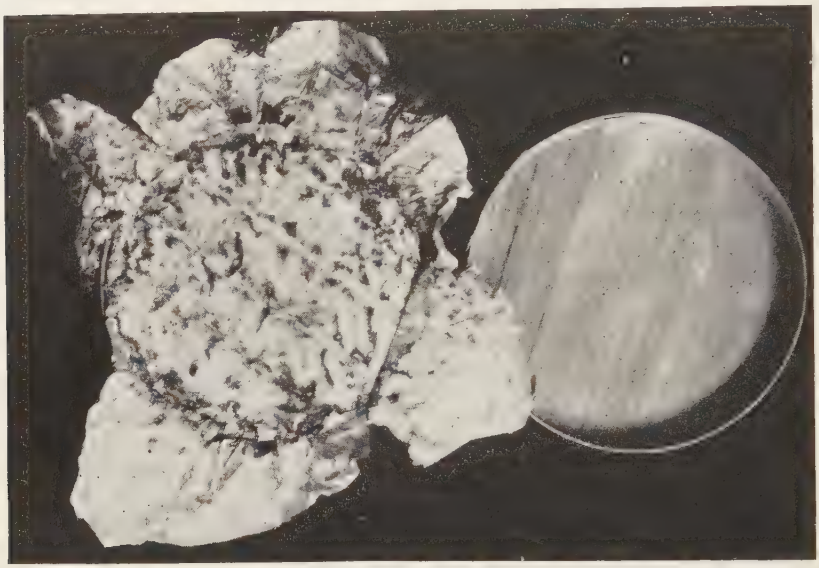


Figure 49. Open package of camembert cheese (domestic) showing normal surface growth.

and *P. camemberti* is not necessary. On this basis precautions are taken to avoid contamination with other molds, and *G. candidum* grows very rapidly at the favorable temperature and high humidity provided. Some of the cheese made in this way is very acceptable to many consumers, whereas others do not regard it as typical camembert.

If *P. camemberti* is to be inoculated on the fresh cheese, it can be grown on water crackers that have been sterilized in a container and then moistened with sterile water to which mold from a stock culture has been added; in some instances 5 or 10% aqueous solution of lactic or tartaric acid is used instead of water. The cheese can be inoculated by sprinkling with, or spraying on, a water suspension of the mold. Kundrat [2] described media for the cultivation of *P. camemberti.* One

consists of sterilized potato chips which are 7 to 10 mm long and the other of parchment paper soaked in sterilized whey. Twice as many conidia developed on the potato medium as on a medium consisting of a mixture of equal parts of whey and a synthetic agar medium. The whey-parchment medium showed the same number of conidia as the whey-synthetic agar medium; the advantage of the former was that the paper could be taken from a petri dish, rolled up with sterile forceps, and mailed in sterilized tubes. Contaminating molds can be detected easily on the parchment paper. The conidia are removed from the paper by shaking in sterile water. Some makers believe it is unnecessary to inoculate after the mold once is established in a plant.

P. camemberti soon is evident on the young cheese and then spreads rapidly over the surface. Later, when spore formation begins, the mold may be still more conspicuous.

Other Surface Organisms. Along with the growth of mold at the surface of cheese there commonly is growth of yeasts and bacteria. *Bact. linens* appears to be of special importance in this connection and the reddish color often noted on the surface of camembert cheese probably is most commonly the result of growth of this species. Eventually, growth of the various organisms results in formation of a slime in which molds are not especially conspicuous.

Esten and Mason [1] determined the numbers of bacteria and yeasts in the slime of cheese, both imported and domestic, and found approximately 7 to 259 billion bacteria per g and 650,000 to 36,000,000 yeasts. In all probability these organisms do not play a necessary part in the ripening process; however, they tend to limit mold growth and may form a coating that partially excludes air and thus aids in controlling off flavors. Conditions provided at the surface of camembert cheese during ripening are very favorable for the development of many species of micro-organisms.

Germination of Spores. Schulz [3] stressed the importance of the germination of the conidia of *P. camemberti* on cheese. High salt concentration interfered with it so germination should take place before the cheese is salted. Germination could be hastened by: (1) Maintaining a temperature of 71.6 to 78.8 F, (2) rapid acid production so as to obtain quick whey drainage and rapid drying of the cheese surface, (3) frequent turning of the cheese in the molds, and (4) use of fresh mold propagations with good vitality and many conidia. Kundrat [4] noted that in the manufacture of cheese it is important that the surface be sufficiently dry and at an acidity favorable for the mold. The growth of yeasts on a moist cheese surface may form a mat which

acts as a barrier to penetration of the mycelia of germinating spores. He [5] also reported that poor mold growth on cheese appeared to be associated with high humidity in the curing room and excessive growth of yeasts at the surface. Laboratory tests indicated that the cultivated camembert mold was unable to germinate on, or penetrate, a film of selective organisms (primarily yeasts) while freshly isolated strains of contaminating molds showed vigorous growth.

Importance of Molds. The necessary mold growth with camembert cheese is limited to the surface. The principal action of the mold is to bring about proteolysis and flavor development. The proteolyzing action of *P. camemberti* can be shown by inoculating sterile curd. Enzymes diffuse from the surface growth and, through their action on casein and certain casein derivatives, bring about pronounced changes that influence the texture and flavor. *P. camemberti* also is active in reducing the acid in the cheese.

Proteolysis in Cheese. The principal proteolytic agents in cheese are the enzymes produced by the surface mold. Bosworth [6] pointed out that the only function of the rennet is to coagulate the milk.

Jacquet and Lenoir [7] reported that, in 2-day-old cheese, 8.7% of the nitrogen was already in soluble form as proteoses, peptones, and peptides. During the ripening the proportion of the soluble nitrogen as peptides increased while that as proteoses and peptones decreased. Free amino acids, negligible in quantity at first, increased after 10 days but never formed 10% of the soluble nitrogen. Ammonia first was detected after 18 days and rapidly increased to form 24% of the soluble nitrogen after 1 month; the cheese then was fully ripe, and about 30% of the nitrogen was in soluble forms. The investigators [8] also noted that in 2-day-old cheese small quantities of aspartic acid, glutamic acid, and leucine were present; at 10 days alanine, arginine, histidine, lysine, tyrosine, and valine also were present; and at 18 days glutamine, methionine, phenylalanine, proline, and hydroxyproline had appeared.

Numbers of Bacteria in Ripened Cheese. Although the maximum numbers of bacteria in cheese are present soon after making and a decrease then begins, there may be large numbers in the cheese mass when ripening is complete. Esten and Mason [1] found that 4 samples of ripened, domestic camembert cheese had approximately 45 million to 2.3 billion bacteria per g and 4 samples of imported cheese had approximately 6 to 442 million.

Cheese from Pasteurized Milk. Hochstresser and Price [9] reported that milk pasteurized at 145 F for 30 min produced cheese of better

average quality and greater uniformity than raw milk. Improvement was greatest when the original milk was of inferior grade. The quality of the pasteurized-milk cheese improved as the quality of the original milk improved. Pasteurization had practically no influence on the yield of cheese.

Defects. Cheese may fail to develop a creamy, waxy texture and remain hard, often with darkening of the cheese mass; presumably, this is due to failure of the cheese to develop the proper proteolytic enzymes in the surface growth. Irregularities in appearance of the surface growth include areas of objectionable color attributable to contaminating molds and other organisms; dark molds [10] and pink yeasts are especially conspicuous. Young cheese may not retain their normal shape because of insufficient acid development. Cheese that is held too long may develop objectionable flavors due to overripening by the normal organisms or to contamination.

Jacquet and Saingt [11] described a defect of cheese which occurred when accelerated ripening was practiced. It was characterized by open texture, cracked surface, and mottled red or brownish-yellow discoloration. The defective cheese had higher nitrate and nitrite concentrations than normal cheese. Also, it contained large numbers of bacteria resembling *Micrococcus conglomeratus*. The micrococci reduced nitrates rapidly. Source of the nitrates in the cheese was unknown. The authors concluded that bacterial reduction of the nitrates caused an evolution of nitrogen and the gas caused the open texture. The nitrites resulting from the reduction reacted with free tyrosine to yield colored nitroso compounds. These could be produced experimentally; the colors obtained varied with the nitrite concentration and *p*H.

Storage of Cheese. Since camembert cheese has limited keeping properties, its proper storage is important. Schulz *et al.*[12] reported cheese could be stored best at 50 to 60.8 F, with proper ventilation. At 68 F, ammonia was formed and the surface became reddish brown due to the predominance of contaminating organisms and formation of whey pockets. When storage was at 23 to 41 F, ripening was delayed and proceeded unevenly causing skin formation and liquefaction in the more rapidly maturing outer layer. The cheese froze at 19.4 to 23 F. Three-quarter-ripe cheese kept well at 10.4 to 14 F for 1 to 4 months, but less mature cheese developed interior cracks and a straw-like texture. For best results, the authors recommended cheese with a moisture content of 68% and a salt content of 3%.

REFERENCES

Camembert Cheese

1. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 83 (1915).
2. *Milchwissenschaft,* **4,** 23 (1949).
3. *Milchwissenschaft,* **2,** 117 (1947).
4. *Arb. Ber. Suddtsch. Vers. u. Forsch. Milchw. Weihenstephan,* **1,** 147 (1950).
5. *Deut. Molkerei-Ztg.,* **72,** 1122 (1951).
6. *N. Y. (Geneva) Agr. Expt. Sta. Tech. Bull.* 5 (1907).
7. *Compt. rend.,* **238,** 2201 (1954).
8. *Compt. rend.,* **238,** 2265 (1954).
9. *J. Dairy Sci.,* **10,** 448 (1927).
10. *U. S. Dept. Agr. Bull.* 1171 (1923).
11. *Lait,* **32,** 129 (1952).
12. *Milchwissenschaft,* **6,** 418 (1951).

COTTAGE CHEESE

Cottage cheese belongs to the group of soft, unripened cheeses in which curdling of the milk is due primarily to acid development, although with most methods of manufacture a small amount of coagulating enzyme added to the milk at setting is a contributing factor. The usual practice is to pasteurize and cool the milk, and then add lactic culture and a small amount of rennet or special coagulator. Often culture that is especially active is employed. The amount of culture added is dependent not only on its activity but on whether a short or long set is to be used; with a set of 3 to 5 hr at 85 to 92 F, 2 to 5% culture may be needed, whereas with a set of 12 to 16 hr at 70 to 80 F smaller amounts are used. When the milk is coagulated and the whey acidity satisfactory, the curd is cut and cooked, the cooking temperature depending on the acid developed, type of cheese desired, etc. After cooking, the whey is drained and the curd washed; the final wash water is well chilled to firm the curd and aid in controlling growth of organisms in it. The flavor is developed largely during coagulation of the milk so the cheese is ready to eat as soon as made. Commonly, cream and salt are added to give a creamed product.

Cottage cheese can be made satisfactorily from reconstituted low-heat skim milk powder, used either by itself or to extend fresh skim milk. In either case, the solids content of the milk used can be kept at about 9% or increased somewhat to get more production per vat.

Sources of Flavor. The flavor of cottage cheese is due to the milk constituents present and to products of growth of the culture bacteria during coagulation of the milk, especially acids. Extensive washing of the curd removes a portion of these products. Some consumers prefer a high flavor, and biacetyl formed during coagulation of the

milk definitely contributes to this. Parker and Elliker [1] found that the desirable flavor of cottage cheese is related directly to the biacetyl content; samples low in biacetyl were typically flat. Biacetyl also can be formed during holding of the cheese, and its odor sometimes is conspicuous. In certain cases addition of a small amount of citric acid to the cheese at creaming has improved the flavor.

Nelson and Brence [2] found no consistent improvement in the flavor of cottage cheese until at least 0.05% citric acid was added to the cheese milk. Before cheese showed an improvement in flavor, it had to contain more than 10 ml 0.1 *N* volatile acid per liter of distillate from 250 g cheese. The volatile acid of cheese was not affected appreciably by diluting the whey with water after cutting the curd or by washing the curd.

Pasteurization of the Milk. In the manufacture of cottage cheese, efficient pasteurization of the milk is important. Without it, the flavor, and also the body and texture, of the product may be greatly influenced by various organisms in the milk that find opportunity for growth during the acid development. Gas-forming organisms, especially *Escherichia-Aerobacter* species, may be so active that the curd is filled with gas holes. Hammer [3] reported gas formation in a vat of milk set for cheese that was so pronounced the gassy curd rose over the edge of the vat. Gas-forming organisms ordinarily produce an undesirable flavor. Recontamination of heated milk, or the curd, also may be serious.

Either LTLT or HTST pasteurization can be used with milk intended for cottage cheese; the latter definitely simplifies an operation in which a number of vats are set. Excessive pasteurization harms the body and texture of the curd.

Importance of the Culture. The culture used is of extreme importance in cottage cheese manufacture since acid development must be reasonably rapid for most satisfactory results. Slow cultures lower the quality of the cheese and upset manufacturing schedules; also, they may cause loss of vats of curd because the quality is so unsatisfactory. Undoubtedly, inadequate cultures are the greatest hazard in cottage cheese manufacture.

Cultures for use in cottage cheese production can be tested for activity with a simple procedure that shows the rate of acid development. Details of the test, such as temperature and per cent culture employed, are determined by the manufacturing procedure followed. If 86 F and 5% culture are being used, an activity test on a culture can be carried out as follows: A small container that can be stoppered

is sterilized and partly filled with an approximate amount (for example, 180 ml) of pasteurized milk which is ready for the cheese vats. The temperature is adjusted to 86 F in a water bath and 5% culture (9 ml for 180 ml milk) added with a sterile pipette and distributed through the milk. The mixture is titrated at once and after 2 and 4 hr, the temperature being maintained at 86 F. The most significant value is the acidity after 4 hr, although the increase in acid from 2 to 4

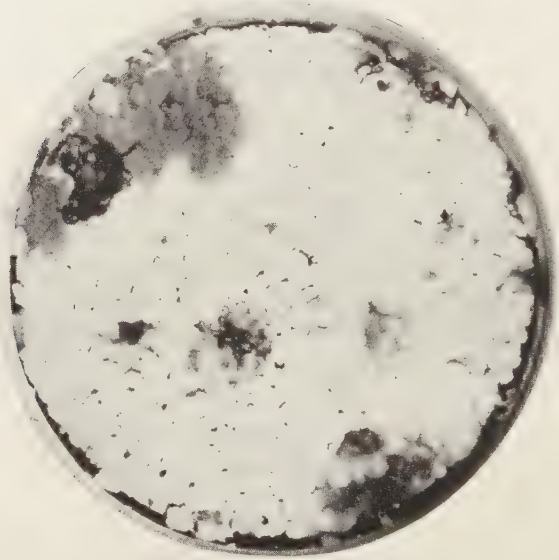


Figure 50. Mold growth on cottage cheese.

hr is important. The culture used for cheese should be active enough to have the curd ready for cutting in the desired period.

Deterioration. The acid present in cottage cheese may inhibit development of certain organisms, but others, especially molds and yeasts, tolerate it, and their growth even may be accelerated by it; the high percentage of water in the product also tends to favor their activity. Accordingly, cottage cheese has poor keeping qualities at the temperatures encountered in some markets and homes.

Molds sometimes appear at the surface of cheese and in air spaces along the wall of the container. There may be many centers of growth with unsatisfactory plant methods or only an occasional one suggestive of air contamination. Color and type of growth vary greatly because of the variety of species involved. *G. candidum* and *Penicillium*

species appear to be the most common. Molds not only spoil the appearance of the cheese but also influence the flavor. Certain of them have pronounced proteolyzing powers and, with time, digest considerable portions of the curd.

Some yeasts develop only where there is an abundant air supply; others are capable of growth through the cheese mass. Considerable surface growth of the species forming white colonies can occur without being especially noticeable. Colonies of pink yeasts are very conspicuous. In general, these organisms have little effect on the flavor of the cheese, even when growth is abundant. A typical yeasty flavor usually is due to the lactose-fermenting species.

Bacteria other than the culture species sometimes are present in cheese in enormous numbers. Their growth may be favored by reduction in the acid at certain points through activity of molds and yeasts. *Ps. fragi* has been found to be the cause of a fruity odor in cottage cheese; it was much more numerous at the surface of the cheese than in the interior.

Parker *et al.*[4] found a gelatinous or slimy curd defect of cheese was caused by three organisms, *Alcaligenes metalcaligenes, Pseudomonas viscosa,* and *Ps. fragi.* The defect was accompanied by a distinct fruity odor and flavor or, in some instances, by a putrid or slightly rancid odor and bitter flavor. The principal sources from which the organisms came were believed to be water used to wash curd and improperly cleaned equipment. Limited control of the defect may be attained by having a low *p*H in the final product, but excluding the causative organisms is the only effective control.

Davis and Babel[5] noted that the rate of slime formation on cheese inoculated with bacteria capable of producing it was related to the temperature of holding. Some cultures failed to produce slime in 14 days at 40 or 50 F and others produced it in 7 to 11 days at 40 F or in 5 to 7 days at 50 F. All cultures produced slime in 1 to 4 days at 70 F. Cheese with a *p*H of 4.7 readily developed slime at favorable temperatures. Curd washed with suspensions containing many bacteria retained from 0.0093 to 0.40% of the organisms. Cultures capable of producing slime on cheese belonged to the genera *Proteus, Pseudomonas, Aerobacter, Alcaligenes,* and *Achromobacter.* All were destroyed at 145 F in 2.5 min. The 2 most resistant cultures survived a 25- but not a 45-sec exposure to 100 ppm chlorine.

In a survey of open market and plant samples of cheese, Foter *et al.*[6] found that the most common spoilage organisms were yeasts which varied in number from 0 to 24,000,000 per g. Of the total samples,

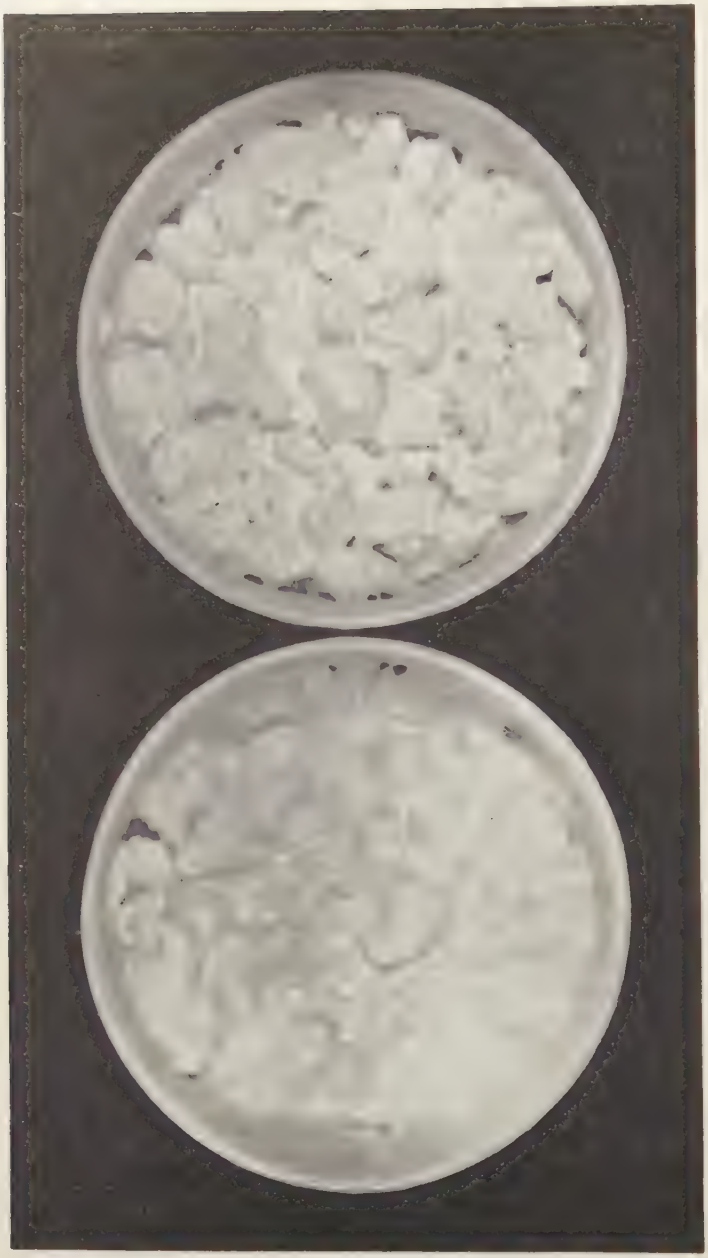


Figure 51. Normal (*upper*) and slimy cottage cheese (*lower*).

31% were yeasty, 22% acidy, and 20% bitter. From their study of retail cheese, Morgan *et al.*[7] concluded that many of the brands included were of poor sanitary quality; 73.4% of the samples had yeast counts of 100,000, or more, per g, 77.3% had mold counts of 10,000, or more, and 2.4% had coliform counts of >100,000. Various flavor defects were encountered, with 28.5% of the samples being bitter, 21.2% acidy, and 22.5% yeasty. A *p*H below 5.0 was not completely effective in controlling the fruity defect due to *Ps. fragi.*

Deane *et al.*[8] reported the flavor criticisms of fresh cottage cheese as acidy or lacking characteristic flavor. Cheese stored at 70 F for 2 days was unclean, fruity, or fermented; that stored at 43 to 45 F for 5 days was acidy, unclean, or bitter. Samples with the highest counts of coliform, lipolytic, and proteolytic bacteria, and yeasts and molds, generally had the poorest keeping quality. Of the organisms studied, molds usually were present in lowest numbers and yeasts in highest numbers. Fruity flavors were due largely to yeasts. The slimy defect was encountered in some samples.

Coliforms in numbers of 220, or more, per 100 g of cheese were found by Lyons and Mallmann[9] in a large percentage of all samples collected. Mechanization of cheese manufacture and packaging was suggested as part of the solution of more sanitary cheese although proper precautions with hand methods may give satisfactory results.

Preservation. The principal method of preserving cheese is through use of low temperatures. It can be held at 32 F for a considerable period without showing significant changes, but at 50 F, or higher, it keeps for a much shorter period. Controlled methods of making and packaging aid in preventing entrance of undesirable organisms and thus have an effect on keeping quality. Cheese curd keeps better than creamed cheese; organisms added with the cream and warming during the creaming may be factors.

Jensen[10] placed fresh cheese curd in brine concentrations from 0.0 to 5.0% and stored it at 45 F. In 3 to 4% brines the curd retained its fresh flavor for 14 days, whereas lower concentrations resulted in flavor deterioration. Curd in fresh water rapidly lost its flavor; that held dry deteriorated most rapidly. The texture of curd stored in 3 to 4% brines was softer and more pliable than that of curd held in brines of lower concentrations. Fouts *et al.*[11] stored cheese curd at 35 to 45 F in 2, 4, and 6% brines. Four per cent brine preserved the curd satisfactorily at 35 F for 2 months, and it was salable as creamed cottage cheese considerably longer. Regardless of the brine concentration, the curd at 45 F was satisfactory for only 1 month; after 2

months mold was present and the curd had a fermented flavor. Storing curd in brine requires a container which will not corrode readily. Olson [12] reported cheese curd can be stored up to 2 weeks at 40 F in 3% brine with little deterioration. Cheese curd sometimes is packed in large containers and frozen so that it can be held for considerable periods. When thawed, the curd is mixed with cream. Commonly, the freezing and thawing break up the curd. The cheese often keeps very well, but off flavors, such as high-acid, stale, bitter, metallic, etc., may develop, owing to growth of organisms before the cheese is frozen or to chemical action.

Laboratory Examination. In order to evaluate the microbiological condition of cottage cheese, it can be examined by the plate method for (1) yeasts and molds, and/or (2) bacteria other than culture types. Ordinarily, all the latter organisms cannot be differentiated by the colony appearance but, if relatively large numbers are present, they are detected easily. Fresh cheese often contains only small numbers of contaminating organisms because the combination of cooking temperature and acid in the curd destroys most of those present when the cooking is begun, even when the numbers are high. However, the product is readily contaminated during handling and, unless kept cold, growth of the contaminants begins.

Cottage cheese that shows flavor deterioration should be plated when it appears organisms are involved. From the appearance of the colonies that develop and/or from microscopic examination of them, the general types of organisms responsible can be determined. Direct microscopic examination of cheese often is helpful in indicating the type of contamination causing a defect.

REFERENCES

Cottage Cheese

1. *J. Dairy Sci.*, **36**, 843 (1953).
2. *Mont. Agr. Expt. Sta. Bull.* 488 (1953).
3. *Ia. Agr. Expt. Sta. Research Bull.* 27 (1927).
4. *J. Dairy Sci.*, **34**, 887 (1951).
5. *J. Dairy Sci.*, **37**, 176 (1954).
6. *J. Dairy Sci.*, **24**, 544 (1941).
7. *Conn. (Storrs) Agr. Expt. Sta. Bull.* 284 (1952).
8. *Am. Milk Rev.*, **16** (5), 60 (1954).
9. *J. Milk and Food Technol.*, **17**, 372 (1954).
10. *N. Dak. Agr. Expt. Sta., Bimonthly Bull.*, **4**, 5, 19 (1942).
11. *Fla. Agr. Expt. Sta. Press Bull.* 593 (1943).
12. *Assoc. Bull. (Intern. Assoc. Milk Dealers)*, **36**, 175 (1944).

PACKAGING FILMS FOR CHEESES

Packaging films are used widely in the cheese industry to protect the products against loss of moisture and to prevent, or limit, surface growth of organisms. They are used on cheese during curing and also to wrap retail portions. The improvements being made in films, in their methods of use, etc., present interesting possibilities.

Curing Cheeses. Cheeses manufactured in blocks are more adaptable to curing in film than other shapes because of the ease of application. However, cheeses in various forms can be cured in films. They may be wrapped in film at the time of the second pressing or after removal from the press; cheeses that were salted in brine also may be placed in film. Pressure and/or heat is used to eliminate air trapped between the film and cheese and to ensure a good seal. With certain films, the air is removed partly by vacuum and the film sealed. Pressure-pack boxes are used extensively in curing to avoid bloating of the films as the cheese produces gases. Normally, a film avoids any appreciable rind formation and the accompanying cutting losses, the cheese cure satisfactorily, and the film is removed easily when the cheese is used. An antimycotic can be added to the cheese surface, or to the film, as further protection against mold growth.

Retail Portions. Wrapping retail portions of cheese in film adequately protects them, limits the moisture loss, permits the purchaser to see the cheese, and eliminates any further wrapping. Between wrapping and display in markets, the portions may be held in pressure-pack boxes. Foil wraps also are used with retail portions of cheese. An antimycotic may be employed with either type of wrap. Process cheese, cheese foods, etc., when still hot, can be packaged in film and sealed.

SPOILAGE IN PROCESS CHEESE PRODUCTS

Process cheese, cheese foods, and cheese spreads constitute an important part of the cheese products marketed. Normally, they keep satisfactorily because of the heat used in processing, acid and salt present, containers which prevent contamination of the finished products, and perhaps other factors. However, spoilage sometimes occurs; it may involve organisms or chemical changes.

Defects in the films used to line boxes or in jar seals may permit the entrance of molds which find conditions suitable for growth. Such

spoilage usually occurs in only an occasional package. Serious spoilage commonly involves heat resistant bacteria which, because of some irregularity, grow in the packaged product.

Griffiths [1] reported instances of spoilage in process cheese by *C. sporogenes* which involved bleaching, development of softened and crumbled areas, and a fecal odor; in some areas there was a small dark slit which was considered to be due to gas. The isolation of *C. sporogenes* and *C. perfringens* from tinned process cheese which was gassy and had a putrid odor also has been reported.[2] The condition was observed only in cheese in which skim milk powder was used. The same types of organisms were isolated from cheese containing no milk powder, but they did not cause deterioration.

No correlation was noted by Csiszar [3] between the acidity of process cheeses and swelling or gas formation. Swelling did not occur at moisture contents below 41%, even with butyric acid bacteria present. Pette and Liebert [4] found that in blown process hard cheese (maximum water content 50 to 55%) lactate-fermenting butyric acid bacteria (*Clostridium tyrobutyricum*) nearly always caused the blowing; sometimes propionic acid organisms did also. In blown process soft cheese (maximum water content 58 to 63%), butyric acid bacteria were responsible although in a few cases putrefactive bacteria played a part. If the maximum processing temperature was below 158 F, blowing occurred, but if higher it generally did not. Possibly, lactic acid bacteria, which can develop with low processing temperatures, caused more anaerobic conditions for the butyric acid organisms.

In two widely separated outbreaks of spoilage in process cheese, in which the cheese was puffed, contained numerous gas holes in the center, and had a putrefactive odor, the causative organism was identified by Hood and Bowen [5] as a variant of *C. sporogenes.* Its main source was skim milk powder used in the cheese, and it was found in about 50% of the powder samples examined. Two factors appeared to be involved in the spoilage, (1) number of the causative organisms present in the process cheese at the time of manufacture, and (2) presence of nutrients supplied by skim milk powder, casein digest, or mature cheddar cheese. Hood and Smith [6] isolated strains of gas-forming clostridia from blown process cheese made with and without skim milk powder and also from cheddar and blue cheeses and from skim milk powder. The defect was produced in process cheese experimentally by *Clostridium pasteurianum, C. sporogenes,* and two unidentified clostridia but not by the other clostridia tried. No defect occurred experimentally in process cheese made with ap-

proximately 4% sodium chloride and a *p*H of about 5.2. Spoilage was more pronounced with larger numbers of spores.

Csiszar and Tomka [7] reported that the periphery of process cheese developed hydrogen 24 hr after wrapping in uncoated aluminum foil. The action was chemical and depth of the affected layer, which gradually developed a doughy consistency, was 2 to 3 mm. Cheese wrapped in lacquer-coated foil did not show the defect. Admixture of a small amount of aluminum dust to cheese resulted in a very gassy condition.

REFERENCES

Spoilage in Process Cheese Products

1. *Queensland Agr. J.*, **52** (Part 2), 186 (1939).
2. *18th Ann. Rept. Council Sci. Ind. Research*, 62 (1944).
3. *Tejgazdaság*, **4**, 1 (1944).
4. *Verslag. Landbouwk. Onderzoek.*, **54** (2), 1 (1948).
5. *Sci. Agr.*, **30**, 38 (1950).
6. *Sci. Agr.*, **31**, 530 (1951).
7. *Intern. Dairy Congr., Proc. 12th Congr.*, **2**, 445 (1949).

SLOW ACID PRODUCTION IN CHEESE MAKING

In making various cheeses, slow acid production is a more or less constant hazard. Certain plants frequently encounter this irregularity; others escape it for long periods and then suddenly have difficulty. Slow acid production varies widely in degree. In some cases acid formation is greatly delayed, whereas in others it is only slightly delayed. With the extreme condition, the vat of milk is lost or is made into low-quality cheese; with only slight delay the important consideration is the longer period required for proper completion of the cheese-making operation.

Bacteriophage. The common cause of slow acid production in cheese making is bacteriophage (Chapter 16). In outbreaks of phage, use of lactic cultures from plants or laboratories that did not supply the one involved in the outbreak is an accepted procedure that repeatedly has restored normal plant operations. If the new cultures are resistant to the strain, or strains, of phage that have become established in the plant, they develop acid actively. Certain plants find it advisable to keep in reserve, in a frozen condition or otherwise, a culture from a new source for use when the regular culture fails.

Phage is retained in cheese and its persistence there may be a factor of importance in outbreaks of slow acid production in plants. Nichols

and Wolf [1] found that several cheese made after the culture had failed or during outbreaks of slow acid production at 3 plants contained phage; concentration of phage was fairly constant when tested periodically during 1 yr. They noted that since phage persists for long periods in cheese it can be carried over from one season to the next.

According to Whitehead and Hunter,[2] the evidence suggests that dormant phage exists in nature and whenever actively growing culture is exposed to chance contamination daily, as in a cheese vat, a particle of appropriate phage eventually falls into the culture, multiplies, and recontaminates the exposed culture as an airborne infection daily. They considered that, if vat surfaces were sterilized immediately before use, multiplication of phage from the air would not be sufficient to check acid development before the cheese was made and that the main danger in cheese making is from the culture used in the vats. Czulak [3] reported that bulk cultures which have coagulated may be contaminated with phage and its action be apparent in the cheese vat. Whitehead and Harkness [4] noted that airborne infection of milk exposed in vats usually is not sufficient to affect acid production before the required acidity is reached; since phage ceases to act at high acidity, it is not significant in cheese making subsequent to salting of the curd.

The effect of adding bacteriophage to the cheese vat after ripening the milk 5 and 30 min has been investigated.[5] With the short ripening, slow acid production was noted during the drying to salting stage. With the longer ripening, acid production was accelerated during early manufacture but this was followed by a decrease in acid production later in the process. With sufficient phage present, acid production was stopped completely and the cheese was of poor quality. Phage infection of liquid milk from air borne particles is reported to be continuous, but airborne phage does not penetrate the curd. With a culture of known activity, ripening before addition of rennet was not considered essential and, from the standpoint of phage contamination, it was regarded as undesirable.[6] Results of Czulak [7] show that a heavy phage infection in the cheese vat after coagulation of the milk has practically no effect on acid development. This was explained by assuming that bacteria in the curd are protected from phage by its inability to diffuse into the curd during the outward movement of whey.

Overby [8] found phage indirectly responsible for the blowing of cheese at a factory having trouble with gas and culture failure. The blown cheese was infected with coliforms and phage, and inhibition

of the culture bacteria by phage enabled the small number of coliforms to multiply. Complete disinfection of the plant and equipment at regular intervals helped control the phage.

Certain investigators consider that slight phage action in a cheese vat, which is not enough to appreciably delay acid production, sometimes modifies the action of the culture organisms (probably the lactic acid type) so that when the cheese is removed from the press it shows small gas holes; ordinarily, these do not increase in size as the cheese is held.

Other Causes. Milk from infected udders and organisms that have an inhibitory effect on the culture bacteria are other possible causes of slow acid production (see Chapter 16). Apparently, these two causes are only occasionally involved and then are responsible for only small delays in acid formation. Because of the peculiar effects sometimes produced by phage, it appears that difficulties due to phage may be ascribed to other causes, such as quality of the milk.

Variations in heat resistance of lactic acid organisms and particularly the influence of various factors on the resistance may result in an unusual effect of the cooking temperature on the activity of the culture organisms. Harrison and Dearden [9] suggested that inability of certain strains of streptococci to grow at cooking temperatures may be one cause of slowness in cheese making. Hunter [10] noted that with some strains of lactic acid organisms variations were well marked, including response to high temperatures.

Penicillin. Hunter [11] added 0.07 to 0.15 unit of penicillin per ml of milk, pasteurized the milk at 155 F, and made it into cheddar cheese. With 0.07 unit, the total time of manufacture was only 5 min longer than the control vat, whereas 0.13 unit increased the total time 55 min. Milk containing 0.15 unit was 1.5 hr slower than the control vat at milling, and no acid was produced thereafter. A comparison was made of the sensitivity to penicillin of single-strain cultures. The most sensitive culture ceased to produce acid soon after draining the whey when 0.07 unit penicillin per ml of milk was present. In milk containing 0.1 unit, 2 cultures required 20 to 30 min additional time for cheese manufacture, while 2 other cultures ceased to produce acid at milling. The most resistant culture (an *S. lactis* type) did not cease entirely to produce acid in milk containing 0.17 unit per ml. Cheese manufactured from milk containing penicillin in sufficient concentration to affect acid production tended to be "short" and "chippy." At 14 days, the cheese were slightly sweet, or sweet, depending on the penicillin concentration. At 3 months, cheese

from vats that were 30 to 40 min slow were weak in body and had an off flavor. Vats in which acid production stopped yielded cheese having a weak, sticky body and rancid and fermented flavors.

Kastli [12] observed early gas production in cheese due to coliforms when the milk contained 0.04 unit penicillin per ml. Mocquot *et al.*[13] noted that cheese made from the milk of cows undergoing treatment with penicillin showed swelling and development of slits after 2 to 3 weeks. The defects were less apparent the day following treatment, and normal cheese resulted 2 days after treatment. Milk from 60 cows, one of which had received an injection of penicillin, gave defective cheese. When made from milk containing penicillin, the cheese had a much higher count of *S. faecalis* than the control cheese.

Activity Tests. Activity tests can be run on lactic cultures intended for various ripened cheeses. They differ from the activity tests on cultures for cottage cheese since ability of the organisms to survive the cooking temperature and retain their acid-producing power is essential. The cheese-making operation [14] can be simulated with a small amount of milk in a sterile container, using all the details of the manufacturing procedure. Conclusions are based on whey acidities, or *p*H values, at various stages in the operation and/or *p*H values on the curd at various ages. The tests permit comparison of a number of lactic cultures at one time and also make it possible to check the activity of the culture used in the operations on different days.

REFERENCES

Slow Acid Production in Cheese Making

1. *J. Dairy Research,* **13,** 302 (1944).
2. *Intern. Dairy Congr., Proc. 12th Congr.,* **2,** 629 (1949).
3. *Australian J. Dairy Technol.,* **7,** 14 (1952).
4. *Australian J. Dairy Technol.,* **7,** 3 (1952).
5. *Australian J. Dairy Technol.,* **7,** 3 (1952).
6. *Australian J. Dairy Technol.,* **7,** 3 (1952).
7. *Australian J. Dairy Technol.,* **7,** 82 (1952).
8. *Maelkeritidende,* **63,** 137 (1950).
9. *J. Dairy Research,* **12,** 35 (1941).
10. *J. Dairy Research,* **10,** 464 (1939).
11. *J. Dairy Research,* **16,** 235 (1949).
12. *Schweiz. Arch. Tierheilk.,* **90,** 685 (1948).
13. *Compt. rend. acad. agr. France,* Jan. 5, 1949.
14. *New Zealand J. Sci.,* **13,** 304 (1932).

VITAMINS IN CHEESES

In connection with studies on the vitamin contents of various foods, many samples of cheeses have been examined. During their manufacture cheeses retain a portion of the vitamins in the original milk, with the remainder, mainly the water-soluble vitamins, being left in the whey. The vitamin contents of certain cheeses are increased through activity of the organisms normally present. Such increases involve soft and semihard types, rather than hard types. Production of vitamins by the organisms developing in, or on, cheeses offers some interesting possibilities in connection with cheese ripening and, eventually, may be considered in developing curing procedures. Hathaway and Davis [1] noted considerable variation in vitamin A contents of 22 kinds of cheeses and also [2] in riboflavin contents of 27 kinds of cheeses and cheese spreads.

Retention of Vitamins. Higuchi *et al.*[3] found that in cheddar cheese making 85% of the vitamin A potency was retained in the cheese, 7% was in the whey, and 8% was not accounted for; the results apply to both carotene and vitamin A. The vitamin A potency of cheese during curing and storage was practically unchanged over almost 1 yr. Guerrant *et al.*[4] reported a measurable decrease in vitamin A potency during ripening of cheddar cheese, but the loss was almost wholly compensated for by moisture losses. Weswig *et al.*[5] found the vitamin A potency of Oregon blue cheese ranged from 5,600 to 9,470 I.U. per lb, depending on the month of production. It appeared that little, if any, of the potency was lost during ripening and storage of the cheese.

Irvine *et al.*[6] noted that 23% of the riboflavin originally in the milk was retained in Canadian cheddar cheese made from raw milk. Riboflavin appeared to be stable throughout 12 months ripening, both at 40 and 58 F. Pasteurization [7] by either the LTLT or the HTST method had no significant effect on retention of riboflavin when compared to raw-milk cheese; values after 8 months ripening were about equal to those of the fresh cheese. With brick cheese [8] 27.4% of the riboflavin originally in the milk was retained in the cheese, and with blue cheese the value was 30.1; riboflavin appeared to be quite stable during the ripening of the cheeses. The pyridoxine content of hard cheese was reported by Debrit [9] to be between 300 and 800 γ per 100 g.

Production of Vitamins. Sullivan *et al.*[10] analyzed 12 types of cheeses for riboflavin, pantothenic acid, nicotinic acid, and biotin.

Only minor variations were noted in the concentrations of riboflavin, but the other factors appeared to be quite dependent upon the age of the cheese and the ripening process involved. Pantothenic acid, nicotinic acid, and biotin values were especially high with camembert and blue cheeses. During ripening of limburger cheese, there was little change in the riboflavin values but a definite increase in pantothenic acid, nicotinic acid, and biotin. Burkholder *et al.*[11] reported that organisms isolated from camembert, liederkranz, brie, and limburger cheeses synthesized considerable quantities of B vitamins in chemically defined media. Thiamin, riboflavin, biotin, and nicotinic acid appeared to increase on the surfaces of these cheeses through progressive stages of ripening.

Todhunter *et al.*[12] determined the vitamin A content of roquefort-type cheese, with and without mold development, and there was no evidence that mold growth increased the vitamin A value. The riboflavin, niacin, and pantothenic acid contents of different cheeses were found to be very variable by Cailleau *et al.*,[13] ranging from 1.8 to 17 γ per g of fresh material for riboflavin, from 0.1 to 23 γ for niacin, and from 0.1 to 11.2 γ for pantothenic acid. Intensity of the fermentation during manufacture of the specific types caused this variation, rather than different lots of milk. Swiss cheese was a poor source of these vitamins while camembert and roquefort cheese were richer sources. Manzini and Artom[14] reported that mold-ripened cheeses had the highest contents of riboflavin, the values ranging from 350 to 453 γ per 100 g. Cheese ripened by lactic acid organisms contained 232 to 269 γ.

REFERENCES

Vitamins in Cheeses

1. *Nebr. Agr. Expt. Sta. Research Bull.* 103 (1938).
2. *Nebr. Agr. Expt. Sta. Research Bull.* 137 (1945).
3. *J. Dairy Sci.*, **29**, 157 (1946).
4. *J. Dairy Sci.*, **21**, 69 (1938).
5. *J. Dairy Sci.*, **34**, 649 (1951).
6. *Sci. Agr.*, **25**, 817 (1945).
7. *Sci. Agr.*, **25**, 833 (1945).
8. *Sci. Agr.*, **25**, 845 (1945).
9. *Intern. Z. Vitaminforsch.*, **24**, 331 (1952).
10. *J. Nutrition*, **25**, 463 (1943).
11. *Food Research*, **8**, 314 (1943).
12. *J. Dairy Sci.*, **25**, 1023 (1942).
13. *Ann. agron.*, **19**, 443 (1949).
14. *Ann. sper. agrar.* (*Rome*), **5**, 805 (1951).

20 Bacteriology of Dairy-Plant Water Supplies

The bacteriological quality of the water that comes in contact with dairy products, or with equipment in dairy plants, is important for two distinct reasons: (1) The water may contain pathogens that are left in the dairy products and/or (2) it may contain organisms that cause spoilage in the products. An additional reason for controlling the bacteriological quality of dairy-plant water supplies is the health of the employees. Dairy-plant employees often drink large volumes of water, particularly when working at relatively high temperatures.

Organisms in water supplies get into a dairy product most readily by direct contact with it. The organisms from such sources that are left on dairy-plant equipment normally are killed when it is sanitized. However, in some instances, attempted sanitization fails through carelessness or the difficulties involved, and the surviving organisms then get into the product when the equipment is used. The danger along this line is greatest with wood equipment, such as churn barrels. With inadequate sanitization of equipment, organisms added to it from water supplies may grow at various points and establish foci from which heavy contamination occurs.

In general, it is not necessary or desirable to rinse before use equipment that has been sanitized with a bactericidal solution; rinsing involves the risk of recontamination with water-borne organisms.

With milk, cream, ice cream, and comparable products, the danger that organisms from the water supply will be present in them is relatively small. Water normally does not come in contact with these products and the equipment used for them is readily sanitized. Should water-borne organisms be included, the usual holding conditions prevent their growth in ice cream and greatly limit it in milk and cream; however, water supplies may contain psychrophiles that grow rather rapidly at temperatures often used for holding milk and cream. With butter and various cheeses, water may be used directly to wash the product, standardize the composition, etc., so that organisms in the water supplies are easily added. As far as spoilage of the product is concerned, the danger is greatest with butter. Various bacteria that cause spoilage in it frequently are present in water, and there often are opportunities for bacterial growth in butter. With cheeses, certain factors tend to prevent growth of many species so that there is less tendency for spoilage from water-borne organisms than with butter; however, such spoilage is by no means excluded, and often the temperature at which cheese is held permits active growth of organisms that tolerate the other conditions.

Water supplies that are satisfactory from the standpoint of public health may not be satisfactory from the standpoint of organisms that cause spoilage in butter, cheeses, etc. The bacteriological methods used in establishing the safety of a water supply for drinking purposes ignore many of the species which can spoil dairy products so that approval of a supply by public health officials means relatively little as to its suitability for butter, etc. Outbreaks of spoilage in butter have been traced to city water supplies that analyses indicated were entirely satisfactory from the public health standpoint.

In a dairy plant a cross connection between a controlled and an uncontrolled water supply is a hazard that should be eliminated because of the possibility of objectionable water getting into the regular supply. Valves that are opened through error, leaky valves, etc., may cause difficulties in this way. The dangers inherent in cross connections commonly are recognized in requirements for water supplies.

The bacteriological quality of a water supply may vary from time to time so that proper supervision requires rather frequent examinations. Variations are of special importance in untreated supplies coming from shallow wells but also involve other types of supplies.

Water as a Source of Butter Spoilage Organisms. Early investigations indicated that organisms coming from contaminated water supplies sometimes cause spoilage in butter. *Ps. fluorescens* received

special attention in this connection, perhaps because it is cultivated easily and produces rancidity in butter. It was isolated from water supplies used in butter plants, and various outbreaks of spoilage were attributed to it.

Outbreaks of cheesiness, surface taint, putrid butter, and rabbito have been traced to contaminated water supplies more frequently than outbreaks of other defects. Herreid *et al.*[1] investigated a plant that had difficulty with cheesy and putrid flavors in butter over a period of 3 yr and found that infected water caused the difficulty; the defect disappeared when a satisfactory water supply was provided. Cullity and Griffin [2] reported that outbreaks of rabbito encountered by various factories could be traced to defective water in every instance and, with improved supplies, the irregularities disappeared. Sorensen [3] examined samples from 22,060 churnings of commercial butter and visited the plants producing butter of poor keeping quality. Putrid and cheesy defects were the ones most frequently encountered, and contaminated water was their main source. Rancid flavors were an important cause of poor keeping quality and they were most frequently traced to equipment, especially churns. Poor keeping quality due to organisms from contaminated water was eliminated by treatment of the water with hypochlorites. Pont [4] reported that the original source of rabbito infection in butter plants appeared to be the water supply.

In various additional investigations flavor defects in butter have been traced to contaminated water. The types of organisms most often responsible are soil and water forms; they include especially organisms belonging to the genus *Pseudomonas* and their ability to grow at relatively low temperatures is significant because of the conditions under which butter often is held.

In experimental churnings there have been many instances of butter spoilage as a result of washing butter with water inoculated with *Ps. putrefaciens, Ps. fragi, Ps. mephitica,* etc. Perhaps the most convincing evidence that contaminated water can cause flavor defects in butter comes from those plants that regularly have spoilage unless the water is treated, usually with chlorine. In some instances neglect of one lot of wash water results in rapid spoilage of the churning involved.

Specific Butter Spoilage Organisms from Water. Organisms that cause spoilage in butter frequently have been isolated from water. Linneboe [5] studied the water supplies of 52 butter plants; 9 contained *Ps. putrefaciens* as the water left the wells and 5 additional as the water left the holding tanks. Of 55 farm-water samples tested, 6 contained the organism. It was detected in water coming from a plant

well 237 ft deep that was drilled through clay and limestone and had the casing well aboveground and set in cement. Long and Hammer [6] found *Ps. putrefaciens* widely distributed in water, including that from streams, lakes, roadside pools, and butter plants. Water from 29 plants was examined and samples from 14 yielded *Ps. putrefaciens* after enrichment in litmus milk. In 8 of the plants the water was suspected of causing poor keeping quality in the butter and *Ps. putrefaciens* was isolated from water of 5 of the 8. With 436 water samples from 70 plants, Corley *et al.*[7] isolated *Ps. putrefaciens* from 22 (5%), representing 14 plants; 105 of the samples yielded typical fluorescent colonies, most of which produced spoilage when added to butter wash water. *Ps. fragi, Ps. graveolens,* and *Ps. mephitica* were obtained from certain samples. White [8] isolated *Ps. putrefaciens* from a dug well water supply that otherwise appeared to be of good quality.

Reynolds and Thornton [9] found 2 cultures of *Fl. maloloris* among 589 isolations of yellow-colony-producing bacteria from 140 samples of Alberta water. Wolochow *et al.*[10] reported that *Ps. fluorescens* was widely distributed in Alberta plant water supplies. Morrison and Hammer [11] isolated *Ps. fragi* from 3 (9.7%) of 31 samples of dairy-plant water.

Investigations in various butter plants have resulted in the isolation from the water supplies of organisms responsible for butter spoilage. Commonly, such isolations have been prompted by consumer complaints and have been followed by treatment of the contaminated supplies.

Water as a Source of Cheese Spoilage Organisms. Organisms that can cause spoilage in certain cheeses are common in water supplies and contaminated water must be looked upon as a possible source. Undoubtedly, the danger is greatest with the washed-curd cheeses. During the ripening of cheeses, conditions permit development of the normal species of organisms so that multiplication of objectionable types also may occur unless they are restrained by such factors as *p*H, salt content, etc., to which the normal species are relatively resistant. Presumably, the acid that develops during the making and early holding of various cheeses is of most significance in this connection. With relatively unfavorable conditions for many species, it is probable that considerable numbers of organisms must be added to cheese for their growth to be extensive enough for spoilage to occur.

With cottage cheese, washing of the curd is prolonged so there is abundant opportunity for its contamination by organisms in the water. The high acidity in the curd presumably prevents growth of certain

species that may come from water, but the limited keeping quality of the cheese shows the great danger of contaminating it with objectionable species. Although the most frequent types of spoilage in cottage cheese appear to be due to acid-resistant organisms, particularly yeasts and molds, other types sometimes are encountered.

Specific Cheese Spoilage Organisms from Water. *Escherichia-Aerobacter* organisms, which are a primary cause of gas formation in various cheeses, often are found in water supplies. In the examination of butter-plant water supplies, they were found frequently; [7] they regularly were absent in the supplies of some plants, regularly were present in the supplies of others, and sometimes were present in the supplies of still others. During the examination of one sample of water from each of 70 plants, 51 isolations of coliform organisms were made; 12 cultures (24%) were *E. coli*, 12 (24%) were *E. freundii*, 16 (31%) were *A. aerogenes*, and 11 (22%) were *A. cloacae*.

Other cheese-spoilage organisms that may come from water supplies are those that produce rancid flavors in cheddar cheese,[12] the nitrate-reducing types that appear to be important in causing discoloration in cheese when potassium nitrate is added to milk to control objectionable fermentations,[13] and *Ps. fragi*.[14] In studies on slimy cottage cheese,[15] *Alc. metalcaligenes* was found in dairy-plant water and *Ps. viscosa* in city water.

Not all the organisms that come from water supplies and develop in, or on, cheeses are objectionable, and certain of them may be useful. *Bact. linens*, which is important on the surfaces of certain semihard and soft cheeses, has been found in surface water and undoubtedly is present in well water. It was obtained rather regularly from 17 samples of water used to water cows or found standing in stables or barnyards and was recovered from 9 of 26 samples of water from streams, roadside pools, and a small lake.[16]

General Studies on Butter-plant Water Supplies. In a number of instances the bacteriological quality of butter-plant water supplies in a given area has been investigated.

Wolochow *et al.*[17] studied 85 samples of water from 37 Alberta creameries. A large proportion of the samples were seriously contaminated with bacteria known to be injurious to the quality of butter. *Ps. fluorescens* was a common contaminant. Results indicated that bacterial contamination which is serious from a quantitative standpoint is likely to be undesirable in qualitative aspects. It was suggested that a short cut to solution of the problem of creamery water

supplies may be to assume that all supplies are likely to contain undesirable bacteria and therefore should be treated.

Study of the water supplies of 42 North Dakota creameries by Jensen [18] revealed that all but 4 contained one or more types of organisms capable of bringing about decomposition of fats and proteins. The organisms usually caused flavor defects in butter when introduced through the wash water. The defects were of various types, with cheesiness being the most common.

Corley *et al.*[7] found, in detailed investigations of water supplies of 70 Iowa butter plants, that some supplies regularly were satisfactory, some regularly were unsatisfactory, and some varied in quality from one examination to another. Certain supplies that were acceptable from a public health standpoint were not suitable for use in butter manufacture because of the presence of butter-spoilage species. When butter was washed with water known to contain coliform organisms, these organisms regularly were found in 1 ml of unsalted butter and usually in 0.1 ml; they sometimes were found in these amounts of salted butter.

Water Storage Tanks. Although many plant water tanks are used without the bacteriological quality of the water being significantly influenced, water coming from such a tank sometimes is poorer in quality than the water going into it. Recent studies [5,7] show that water tanks may be sources of coliform and/or characteristic butter-spoilage species. Presumably, wood tanks are more often a source of butter-spoilage organisms than metal tanks. Organisms falling into a poorly covered tank may be responsible for such contamination, but growth also is involved, particularly when there is opportunity for organic matter to accumulate in the tank.

Castell and McDermott [19] found that water from several hundred deep and shallow wells, having low initial counts, contained organisms that multiplied extensively when the water was enclosed in a container. The organisms included mainly types having the general characters of the genera *Alcaligenes*, *Pseudomonas*, and *Achromobacter*. A large proportion of them were lipolytic, proteolytic, and produced oxidase; many were active at low temperatures. Inoculation of sterile tap water with pure cultures appeared to confirm the observation that growth in water of low organic content was restricted to certain groups of gram-negative bacteria, especially *Pseudomonas* species.

A water storage tank should be recognized as a possible source of organisms in a water supply and adequate protection and care should be given it. Bacteriological tests of the water going to, and coming from, the tank are advisable.

Attempts to Detect Butter-Spoilage Organisms in Water. Most of the studies on spoilage organisms in dairy-plant water have been carried out from the standpoint of butter because the dangers are greatest with this product. Both quantitative and qualitative considerations are involved since various factors influence growth of organisms in butter, and wash water may contain spoilage organisms without causing deterioration. In salted butter with desirable physical properties, heavily contaminated wash water *may* not cause spoilage. Even with unsalted butter having physical properties that favor growth of organisms, considerable numbers of spoilage types *may* be required to cause a defect.

Butter Washing Tests. One type of test employed on water involves actual washing of small lots of experimental unsalted butter (made from pasteurized cream with sterile equipment) with the water in question, and holding the butter at favorable growth temperatures, such as 70 F. Rapid spoilage of the butter, when the control butter washed with water from the same lot after sterilization keeps satisfactorily, is convincing evidence of the danger involved. Delayed spoilage may be due to irregularities of one type or another and is much less convincing than rapid spoilage. In general, butter washing tests have been helpful in the detection of butter-spoilage organisms in water, especially when the species involved grow poorly, or not at all, on the media used to plate the water.

Plating Procedures. Because the organisms causing spoilage in butter vary greatly in colony type, action on protein, action on fat, etc., and also because nonspoilage species frequently show characters essentially the same as those of certain spoilage species, detection of spoilage organisms by the plating of water presents many difficulties, even with differential media. The problem is further complicated by the fact that such an important spoilage species as *Ps. putrefaciens* does not initiate growth readily on media, including those especially favorable for it.

In general, actively proteolytic and/or lipolytic bacteria are objectionable in a water supply. However, it should be recognized that not all organisms showing one or both of these characters produce changes in butter. With 64 oxidase-positive and 28 oxidase-negative cultures, Trembath [20] found that the former produced spoilage in butter much more commonly than the latter but the correlation was not complete.

Plating of water samples, particularly on a medium permitting detection of proteolysis and lipolysis, supplies useful general information with reference to a water supply and is especially helpful in detecting

changes in the bacteriological condition of a supply. The plates should be incubated at relatively low temperatures, such as 70 F (21.1 C), since certain significant organisms fail to grow at somewhat higher temperatures. By incubating plates at various temperatures, additional information may be obtained. Jezeski and Macy [21] reported that some samples of creamery water yielded bacterial counts of considerable magnitude when the plates were incubated at 46.4 F (8 C).

Significance of Total Plate Counts. It would be expected that the higher the bacterial count on butter wash water, the greater the danger from it. This general relationship is shown by the following data reported by Corley *et al.*[7] The counts were made on tryptone glucose extract (milk) agar, the plates being incubated 4 days at 69.8 F (21 C), and the effect of the water on butter washed with it was tested in churnings, each involving about 1 pt of cream; the butter was unsalted.

General Relationship Between Numbers of Bacteria in Butter Wash Water and Production of Flavor Defects in the Unsalted Butter

Group	Bacterial Counts on Water — Range per Milliliter	Number Samples in Group	Samples of Water Producing Flavor Defects in Butter in 4 Days or Less (per cent)
a	<1 to 50	204	7
b	51 to 100	43	23
c	101 to 200	52	44
d	201 to 300	31	32
e	301 to 400	22	50
f	401 to 750	35	47
g	751 to 1,500	36	61
h	over 1,500	13	69

The results show that a small percentage of low-count samples caused spoilage, and some of the high-count samples failed to do so. The former irregularity presumably was due to failure of certain species important in butter spoilage to develop colonies on the plates; the latter irregularity evidently was because certain species of bacteria do not cause spoilage even when present in considerable numbers.

Bacterial Standards for Dairy-plant Water Supplies. Bacterial standards for dairy-plant water supplies should recognize the two general groups of organisms that are objectionable from the standpoint of dairy products: (1) Those of public health significance, and (2)

those that may be important in causing spoilage in dairy products. Although spoilage due to contamination from water supplies is less common with various other dairy products than with butter, there is little justification for using supplies in the preparation of such products that would not be acceptable in butter plants. Moreover, contamination of market milk, market cream, etc., with the types of organisms involved in butter spoilage may cause deterioration in them should the holding conditions be favorable for growth as a result of carelessness in the producing plants, distribution centers, homes, etc. Spoilage of market milk and cream with such typical soil and water organisms as *Ps. fragi, Ps. fluorescens,* etc., is rather common, even with moderate holding periods.

Based on earlier suggestions,[7] the following standards are proposed for butter-plant water supplies and probably also should be applied to all types of dairy plants.

1. The water should meet all drinking water standards.

2. The total bacterial count on agar (standard for milk) containing fat emulsion and skim milk, with incubation 4 days at 69.8 F (21 C), should not exceed 100 per ml and preferably should be under 50.

3. If proteolytic and/or lipolytic bacteria are present, the numbers should be relatively low, and actively proteolytic or lipolytic organisms, as indicated by the colony types, should not exceed 5 per ml.

4. When the water is used to wash butter in experimental churnings, the unsalted butter should keep satisfactorily for at least 4 days at 69.8 F. Experimental churnings are not needed in regular examinations of water supplies but are advisable when the quality of the water is still in doubt after the usual tests or when a supply definitely is suspected of causing deterioration in butter.

5. A water supply should be examined at regular intervals; if it commonly is satisfactory, examinations can be less frequent than if it is variable in quality.

Water Treatment. Recognition of the role of contaminated water in the spoilage of dairy products has led to various suggestions with reference to the treatment of water of unsatisfactory bacteriological quality. For the most part the treatment has been considered necessary because of the presence of spoilage organisms, but if species of public health significance are present they also will be included.

Pasteurization and recooling of water has been advocated and undoubtedly is effective. Such treatment may be applicable to a small operation as an emergency procedure but is expensive and inconvenient; for a large operation it is impossible without special equip-

ment and even then the expense eliminates it as a practical procedure. Filtration through special filters, light treatment, ozone treatment, various chemical treatments, etc., also have been proposed. Treatment with chlorine is the most widely used procedure and its effectiveness, low cost, convenience, etc., have been established. Various types of equipment have been developed for introducing the chlorine, in one form or another, into the water. The extensive use of chlorine in the treatment of city water supplies is ample evidence of its value.

Sediment in Water. In some instances water contains excessive amounts of sediment which can be added to a dairy product directly or left in equipment so that it then gets into a product when the equipment is used. Water of this type may or may not be satisfactory bacteriologically. Filters of various types commonly are employed to remove such material; when used as the water is flowing from pipe lines into churns, vats, etc., they are not always effective because, with the speed at which the filters must operate, removal of finely divided material is difficult. With certain water supplies, filtration and/or sedimentation under conditions which favor rather complete removal of sediment may be desirable.

Because of the attention given extraneous matter in dairy products, sediment in water should be removed as completely as possible. Windblown material that gets into water directly, or by first settling into open equipment, also should be considered.

REFERENCES

1. *Minn. Agr. Expt. Sta., Tech. Bull.* 97 (1934).
2. *J. Dept. Agr. W. Australia,* **15,** 137 (1938).
3. *J. Dairy Sci.,* **23,** 423 (1940).
4. *J. Council Sci. Ind. Research,* **14,** 1 (1941).
5. *Sci. Agr.,* **21,** 133 (1940).
6. *Ia. Agr. Expt. Sta., Research Bull.* 285 (1941).
7. *Ia. Agr. Expt. Sta., Research Bull.* 319 (1943).
8. *Can. Dairy Ice Cream J.,* **22** (4), 27 (1943).
9. *Sci. Agr.,* **24,** 21 (1943).
10. *Sci. Agr.,* **22,** 637 (1942).
11. *J. Dairy Sci.,* **24,** 9 (1941).
12. *Can. Dept. Agr. Bull.* 146 (1931).
13. *Can. J. Research,* **1,** 256 (1929).
14. *B. W. Hammer Panegyric,* 123, 1937. Collegiate Press, Inc., Ames, Ia.
15. *J. Dairy Sci.,* **34,** 887 (1951).
16. *Ia. Agr. Expt. Sta., Research Bull.* 328 (1944).
17. *Can. Dairy Ice Cream J.,* **20** (2), 23 (1941).
18. *N. Dak. Agr. Expt. Sta. Bimonthly Bull.,* **4** (5), 18 (1942).
19. *Food Research,* **7,** 244 (1942).
20. *J. Australian Inst. Agr. Sci.,* **8,** 150 (1942).
21. *J. Dairy Sci.,* **29,** 439 (1946).

Index

Absorption of odors by milk, 27
Accelerated ripening, of blue cheese, 565
  of cheddar cheese, 536
Acetic acid production, by S. *lactis,* 73
  in lactic cultures, 380
Acetylmethylcarbinol, in butter, 461
  in lactic cultures, 375, 380, 382
Achromobacter lipidis, 102
Achromobacter lipolyticum, 102, 501
Acid, effect in butter, 466, 475
Acid-forming organisms, counting, 15
Acidity of fresh milk, 31, 66
  changes in, 66
Acidophilus milk, 435
  viability of bacteria, 436
Acids, *see* Washing reagents
Acids in cheddar cheese, 523
Actinomyces, 104, 333
Activity tests on lactic cultures, 579, 590
Advanced lactation, effect on milk flavor, 28
Aerobacter, see Escherichia-Aerobacter
Aerobacter aerogenes, 104, 122, 163, 597
Aerobacter cloacae, 341, 597
Agar disc method, 130
Alcaligenes metalcaligenes, 581, 597
Alcaligenes viscosus, 91, 94
  var. *dissimilis,* 91
Alcohol test, 32
  conditions causing coagulation, 33
  modifications, 33
Alkalies, *see* Washing reagents
Alkyl-dimethyl-benzyl ammonium chloride, *see* Bactericidal reagents
Anaerobic spore test, 45
Anthrax from milk, 266
Antibiotics, effect on lactic cultures, 402
  extent in milk, 403
Aseptically drawn milk, numbers of bacteria in, 117
  types of bacteria in, 120
Aspergillus repens, 333
Aspergillus glaucus, 333
Associative action, 64
Aureomycin as milk preservative, 171
Avoset, 325

Bacillus albolactis, 99
Bacillus amarus, 322
Bacillus anthracis, 266
Bacillus calidolactis, 82, 320

Bacillus cereus, 99, 305, 321
Bacillus coagulans, 82, 319
Bacillus megatherium, 317, 320
Bacillus mesentericus, 305, 321
Bacillus metiens, 161
Bacillus panis, 322
Bacillus polymyxa, 552
Bacillus putrificus, 553
Bacillus simplex, 320
Bacillus subtilis, 99, 163, 165, 319, 321, 403
Bacillus vulgatus, 320
Bacterial counts on dairy products, 3
  differential media, 15
    acid-forming organisms, 15
    lipolytic organisms, 16
    proteolytic organisms, 15
  field procedures, 23
  microscopic, 4
  plate, 7
  routine, 3, 4
  significance, 10, 12
  special, 3, 14
Bacterial growth in raw milk, 174
Bacterial standards, milk and cream, 13
Bactericidal action of milk, 182
  causes, 184
  effect of heat, 183
  significance, 186
Bactericidal reagents, 160
  action on bacteria, 163
  addition to cleaners, 164
  compounds used, 160
  corrosive action, 164
  factors influencing, 161
Bacteriophage in lactic cultures, 407
  control, 416
  description, 408
  destruction, 413
  estimation, 409
  multiplication, 412
  nascent phenomenon, 419
  relationship to bacteria, 410
  sources, 415
Bacterium linens, brick cheese, 556
  camembert cheese, 575
  limburger cheese, 571
Biacetyl, in butter, 375, 458, 461
  in lactic culture, 381
Bichloride of mercury as milk preservative, 172
Bitterness following proteolysis, 101
Blue fermentation, 106
Blue-veined cheeses, action of mold on acids, 562
  bacteriology, 558
  bleaching fat for, 559
  defects, 566
  factors influencing mold, 564
  factors influencing ripening, 565
  flavor, 563
  lipolysis, 562
  milk used, 558
  mold, 559
  mold powder, 559
  proteolysis, 561
Body cells in milk, 51
  influence of condition of udder, 55
  methods of counting, 52
  numbers, 52
Bottle caps, 238
Brick cheese, bacteriology, 554
  defects, 557
  organisms in, 554
  salting, 555
  smear, 556
Brucella abortus, 259
Brucella melitensis, 259
Brucella suis, 259
Brucella organisms in cows' milk, 260
  dangers from, 261
  effect of heat, 205
  survival, 284
Brucellosis, human cases, 259
  control from dairy products, 262
Buddeized milk, 169
Bulgarian milk, 435
Bulk handling of milk, 150, 178
  bacterial counts, 152
  cleaning tanks, 152
  problems with, 153
Bulk milk dispensing, 248
Burri smear culture technic, 20
Butter, bacteriology, 442
  butyric acid, *see* Butyric acid
  control of microbiological defects, 484
  culture, 373

Butter, deterioration, 463
bacterial action, 463
chemical action, 465
enzymatic action, 464
determination of bacteria, 443
determination of yeasts and molds, 445
effect of organisms in, 457
factors limiting bacterial growth, 452
mold hyphae, *see* Mold hyphae
moldy, 485
growth of mold, 486
mold inhibitors, 488
sources of mold, 485
numbers of bacteria in fresh, 451
*p*H determinations, 503
phosphatase tests, 504
sectional contamination, 484
sources of organisms, 447
special cultures for, 422
specific flavor defects, 469
due to cream, 469
due to plant methods, 471
that develop, 472
cheesiness, etc., 475
fishiness, 473
rancidity, 472
roquefort flavor, 484
tallowiness, 480
unclean flavors, 483
standards for yeasts and molds, 447
surface discoloration, 490
due to sulfur dioxide, 491
due to desiccation, 492
survival of pathogens, 285
tests for keeping quality, 504
water-insoluble acids, *see* Water-insoluble acids
Buttermilk, preparation, 431
Buttons, sweetened condensed milk, 333
Butylene glycol, in lactic cultures, 381
Butyric acid, in cream and butter, 503

Camembert cheese, bacteriology, 573
defects, 577
germination of spores, 575
molds, 573
media, 574
Camembert cheese, organisms, 573, 576
pasteurization of milk, 576
proteolysis, 576
storage, 577
surface organisms, 575
Candida krusei, 545
Candida pseudotropicalis, 83, 470, 552
in alcohol production, 108
in production of yeast, 109
isolation, 85
Canned fresh milk, 325
Cans returned to farms, 244
Carbon dioxide, in gassy fermentation, 82
in lactic culture, 380
Carbonation of butter, 469
Catalase, 310
of bacteria, 311
Catenularia fuliginea, 333
Cheddar cheese, accelerated ripening, 536
action of salt, 523
bacteriology, 515
changes in numbers of bacteria, 517
chemical compounds in, 523
clarification of milk, 535
color changes, 530
defects, 525
gas, 525
lack of acid, 525
moldy, 532
inhibitors, 533
off flavors, 528
openness, 527
importance of acid development, 516
importance of *p*H, 523
organisms, 516, 518, 520
pasteurized-milk cheese, 516
protein decomposition, 521
raw-milk cheese, 515
white particles, 525
Cheeses, bacteriology, 510
classification, 511
determination of organisms, 513
distribution of organisms, 513
general ripening agents, 512
plant sanitation, 515
quality of milk, 515

Cheeses, slow acid production, 587
bacteriophage, 587
other causes, 589
penicillin, 589
survival pathogens, 286
vitamins, 591
Cheesiness, butter, 475
cream, 470
Chemical action, in butter, 465
in milk, 29
in sweetened condensed milk, 334
Chlorine compounds, *see* Bactericidal reagents
Cholera, from dairy products, 281
Chromobacter violaceum, 403
Churns, care, 449
contamination, 448
Circulation cleaning, 241
control procedures, 243
HTST pasteurizers, 243
pipe lines, 241
storage tanks, 243
Citric acid, addition to lactic culture, 382
breakdown, 385
Cladosporium butgri, 473
Clarification, 193
amount of slime, 193
composition of slime, 193
effect on bacteria, 194
effect on body cells, 195
effect on dirt, 196
effect on keeping quality, 196
for cheddar cheese, 535
for swiss cheese, 543
full clarifier bowl, 196
Clean cows, influence on bacterial count, 127
Clipping udders, influence, 129
Clostridium botulinum, 283
Clostridium genus, 45, 89
Clostridium pasteurianum, 586
Clostridium perfringens, 552, 586
Clostridium sporogenes, 46, 586
Clostridium tyrobutyricum, 586
Coat of cow, material from, 125
reducing contamination from, 127
Coliforms, *see Escherichia-Aerobacter*
Color fermentations, 105
Consumer complaints, 247
Cooling and holding milk on farms, 176
bacteria in fat layer, 180
cooling in air, 178
cooling in water, 176
delivery uncooled milk, 179
effect delayed cooling, 180
Copper, tallowy butter, 481
Copper sulfate to detect lipolysis, 17
Corynebacterium diphtheriae, effect of heat, 206
survival in milk, 284
Cottage cheese, bacteriology, 578
deterioration, 580
laboratory examination, 584
lactic culture, activity test, 579
importance, 579
pasteurization of milk, 579
preservation, 583
source of flavor, 578
Cow manure, bacteria in, 126
detection of fragments, 126
Coxiella burnetii, in milk, 263
effect of heat, 207
Cream for butter, care in plants, 494
care on farms, 493
production, 492
separation, 492

Dairy bacteriology, importance, 1
Dairy plant employees, examination, 288
Dehydroacetic acid as mold inhibitor, 490, 533
Delayed cooling of milk, 180
Destruction of organisms by pasteurization, 203, 289
Dichlorodimethylhydantoin, 161
Differential media, plate counts, 15
Diphtheria from dairy products, 280
Diplococcin, 420
Dirt in milk, 125
removal by clarification, 196
removal by filtration, 191
Diseases, decrease in spread through dairy products, 253
groups spread through dairy products, 254
Drying, effect on bacteria, 343
effect on lactic cultures, 388

Dry milk, *see* Milk powder
Dye reduction tests, 20
Dysentery and diarrhea from dairy products, 276

Electrical treatment of milk, 228
Emmenthaler cheese, *see* Swiss cheese
Employees, contamination from, 135, 240, 450, 515
Enzymes of milk, 294
Epidemics, milk-borne, characteristics, 271
Equipment, care, 133, 234, 240
  contamination from, 129, 235
    methods of measuring, 129
  growth in, 132
  influence of sterilization, 134
  organisms from, 130
  sanitizing before use, 134
Escherichia-Aerobacter organisms, counting, 19
  division of group, 85
  growth in milk, 87
  importance in dairy products, 87
  in butter, 463
  in cheese, 525, 578
  in evaporated milk, 323
  in gassy fermentation, 83
  in milking machines, 141
  in sweetened condensed milk, 331
  isolation from dairy products, 88
  presence in milk, 12
  ropy strains, 92
  significance in milk, 47
  sources in milk, 47, 86
  survival during pasteurization, 218
Escherichia coli, 41, 163, 165, 199, 360, 365, 597
Escherichia freundii, 597
Evaporated milk, bacteriology, 314
  examination, 314
  factors influencing spores, 316
  incubation, 318
  spoilage, bitterness, 322
    coagulation, 319
    control, 324
    due to defective cans, 323
    gas formation, 321
  sterilization exposures, 316

Fat, action of bacteria on, 102
  changes in butter, 481
  in detecting lipolysis, 16
Fat layer of milk, bacteria in, 180, 245
Feed flavors, in butter, 470
  in milk, 27
Fermentation cycle, raw milk, 67
Fermented milk preparations, bacteriology, 431
  holding, 440
Filtration of milk, 132, 189
  contamination from filters, 192
  effect on bacteria, 190
  general effect, 190
Fishiness in butter, 473
Flat flavor, butter, 472
Flavobacterium maloloris, 480, 596
Flavor fermentations, 103
Flavor organisms of lactic cultures, 376, 422
  action on citric acid, 385
  action on protein, 387
  distribution, 378
  in ropy cultures, 395
  isolation, 377
Flavors in milk, due to organisms, 103
  due to other causes, 27
Flies, organisms from, 135
Foam on milk, bacterial count, 209
  temperature, 209
Foot-and-mouth disease, from milk, 264
Foremilk, counts on, 119
  effect of elimination, 119
Formaldehyde, *see* Preserving reagents
Freezing, concentrated milk, 349
  concentrated skim milk, 351
  cream, 357
  effect on bacteria, 347
  homogenized milk, 348
  milk, 347
Frost little plate method, milk, 22
  ice cream, 356
Frozen-dried lactic cultures, 389

Galactase, 305
Gargety milk, 89
Gassy fermentation, 82
  causative organisms, 83
  importance, 83

Geotrichum candidum, 109, 473, 495
destruction by heat, 111
in biosynthesis of fat, 111
in lactic cultures, 424
in milking machines, 141
on camembert cheese, 573
on limburger cheese, 572
Glass milk bottles, 236
Gorgonzola cheese, 558
Gray decay in swiss cheese, 552
Gray discoloration in blue cheese, 567
Growth and longevity of pathogens, 284
Growth of organisms, in butter, 457
factors influencing, 452
in pasteurized milk, 221, 246
in raw milk, 174, 186

Hand milking, sources of organisms, 123
Heat resistance bacteria, factors influencing, 211
Heat resistance pathogens, 204
High acid flavor, butter, 472
High temperature pasteurization, 202
Holding milk, *see* Cooling and holding milk
Homogenization, 198
effect on clumps of bacteria, 198
contamination, 199
Homogenized milk, counts on, 199
for blue cheese, 558, 565
for cheddar cheese, 539
pasteurization, 200
Hormodendrum olivaceum, 568
Hotis test, 57
Hypochlorites, *see* Bactericidal reagents
Hydrogen peroxide, 169, 310, 325

Ice cream, bacteriology, 353
bacteria from equipment, 362
bacteria from ingredients, 358
bacterial standards, 370
changes in numbers of bacteria during manufacture, 363
coliform counts, 356, 360, 361
contamination, from air, 362
from containers, 370
from dippers, 369
from vendors, 369
Ice cream, determination of numbers of bacteria, 353
Burri technic, 355
Frost little plate, 356
plate, 354
microscopic, 354
hardening, 367
numbers of bacteria, 357
products related to, 370
storage, 368
survival of pathogens in, 285
types of bacteria in, 357
Increased acidity in milk, detection, 30
Inspection, *see* Supervision of milk supplies
Interior of udder, bacteria from, 117
Intestinal disturbances in children, 281
Iodophors, 167

Jimmy weed, 268
Johne's disease, from milk, 265

Keeping quality of milk, effect of clarification, 196
effect of pasteurization, 224
Keeping quality tests, on butter, 504
on milk, 34, 247
Kefir, 437
Kumiss, 438

Lactenin, 184
Lactic acid, 65
fermentation, 65
formation from lactose, 66
optical isomers, 66
organisms, 68
preservative action, 67
Lactic cultures, activity tests, 425, 579, 590
addition of citric acid, 382
air supply, 401
antibiotics in, 402
aureomycin, 402
penicillin, 402
bactericides in, 407
bacteriology, 373
bacteriophage, *see* Bacteriophage
citric acid breakdown, 385
compounds formed, 379
developing, 378

Lactic cultures, factors influencing acid production, 397
history, 374
holding, 385
incubation temperature, 384, 400
inhibition by organisms, 420
judging, 395
laboratory examination, 423
large volumes, 394
milk, heat treatment, 399
mother cultures, 390
numbers flavor organisms, 384
overripening, 400
oxygen supply, 383
*p*H, 382
propagation, 387
frozen-dried cultures, 389
heat-dried cultures, 388
liquid cultures, 388
protein breakdown, 387
Lactobacillus acidophilus, 79, 435
Lactobacillus brevis var. *rudensis*, 531
Lactobacillus bulgaricus, 79, 93, 435, 439, 520, 545
Lactobacillus casei, 79, 93, 519, 545
Lactobacillus enzymothermophilus, 305
Lactobacillus helveticus, 79, 545, 553
Lactobacillus organisms, 77
in dairy products, 78, 81
isolation, 81
species important, 79
sources, 81
Lactobacillus plantarum var. *rudensis*, 531
Lactobacillus thermophilus, 213
Lactose, transformation to lactic acid, 66
Lecithin, decomposition in butter, 474
Leucocytes, *see* Body cells
Leuconostoc citrovorum, 377
Leuconostoc dextranicum, 377
Limburger cheese, bacteriology, 569
defects, 572
gas production, 571
pasteurization of milk, 571
smear, 570
streptococci, 569
Line tests, 24
Lipase, 295
Lipase, estimation, 295
factors decreasing activity, 298
factors increasing activity, 296
inactivation by heat, 299
in cream, 300
in mammary tissue, 300
relationship of animal to activity, 300
Lipolysis, by lipase, 295
by organisms, 101
Lipolytic organisms, counting, 16
Low-temperature long-time pasteurization, 202

Machine milking, 139
Malty flavor, *see Streptococcus lactis* var. *maltigenes*
Mastitis, 269
causative organisms, 269
extent, 270
importance in human disease, 270
spread, 270
Metallic flavor, butter, 470
Metals in butter deterioration, 467
Methyl ketones in blue-veined cheeses, 563
Methylene blue test, 35
bacteria present at reduction, 38
comparison with resazurin test, 43
effect of tube inversion, 36
influence of penicillin, 44
influence of various factors, 36
mechanism of reduction, 37
variations in organisms, 35
Microbacterium flavum, 214
Microbacterium lacticum, 214
Microbacterium liquefaciens, 214
Micrococcus candidus, 213
Micrococcus caseolyticus, 122, 213
Micrococcus conglomeratus, 577
Micrococcus cremorisviscosi, 92, 94
Micrococcus epidermidis, 213
Micrococcus freudenreichii, 93, 122, 522
Micrococcus luteus, 213
Micrococcus pituitoparus, 92
Micrococcus pyogenes var. *aureus*, 58, 168, 199, 269, 283, 329
Micrococcus varians, 213
Microscopic colony count, 22

Microscopic count, 4
advantages, 4
comparison with plate count, 9
disadvantages, 6
effect of pasteurization, 6
preserving samples, 6
Milk abnormalities, characteristics of bacterial, 113
Milk control, accomplishments, 289
Milk enzymes, 294
Milk fermentations, 63
influence of temperature, 175
mixed, 64
normal and abnormal, 63
Milk plant operation, 234
cans returned to farms, 244
handling raw milk, 235
pasteurization, 235
selection of milk, 234
Milk plant processes, 189
Milk powder, bacteriology, 336
bacterial standards, 340
changes in numbers of bacteria, 341
effect of humidity, 342
effect of drying on organisms, 343
keeping quality of reconstituted, 345
microscopic count, 338
plate count, 337
organisms, numbers, 339
types, 341
relationship of organisms, 344
significance of counts, 342
Milk production, bacteriology, 116
Milk sickness, 267
distribution of cases, 269
plants responsible, 267
toxic constituents, 268
Milk, survival of pathogens, 284
Milk-borne epidemics, characteristics, 271
methods of tracing, 273
Milker, contamination from, 135
Milking machines, bacteria in, 140
care, 142, 147
chemical treatment, 142
flora, 141
heat treatment, 144
holding in water, 145
importance of clean udders, 147
Milking machines, precautions in studying care, 145
special treatments, 146
Milking parlors, 150
Molds, counting, 19, 445
in milk, 109
Mold hypae, in butter, 497
compared to cream, 497
compared to flavor, 497
determination, 497
in cream, 495
determination, 495
factors influencing, 495
Mold inhibitors, for butter, 488
for cheese, 533
Mycobacterium tuberculosis in milk, 163, 170, 256, 257, 344
effect of heat, 204
examination, 256

Neutralization of cream, 467
Neutralizer flavor, butter, 472
Nile blue sulfate, to detect lipolysis, 17, 18
Nisin, 421
Nonacid milk, 420

Oidium lactis, 109
Old cream flavor, butter, 470
Oospora lactis, 109
Organisms required to produce changes, 111
Ortho-mercurate, 172
Oval tube technic, 21
Oxidase, 311
bacteria-producing, 311
Oxidase-producing bacteria, detection, 18
Oxidation, butter, 481
butterfat, 482
frozen milk, 349

Packaging films for cheese, 585
Paper bottles, 238
Paratyphoid, from dairy products, 276
Pasteurization, 201
advantages, 223
bacteria surviving, 210
bacterial efficiency, 203

Pasteurization, basis of exposures, 203
comparison of LTLT and HTST, 220
cooling after, 210
effect on pathogens, 204
factors influencing resistance of organisms, 211
home, 210
importance of uniform heating, 208
influence of foam, 209
influence of pellicle formation, 208
low bacterial efficiencies, 216
margin of safety, 208
objections raised to, 224
origin of, 201
special time-temperature relationships, 227
types, 202
Pasteurized milk, bacterial growth in, 221, 246
fermentation, 221
Pathogens, in dairy products, 252
preventing spread, 289
survival, 284
Penicillin, duration in udder, 405
effect on dye reduction, 44
effect on lactic culture, 402
in cheese, 589
see Preserving reagents
stability, 406
Penicillinase, 44, 403
Penicillium camemberti, 573
Penicillium glaucum, 559
Penicillium oxalicum, 564
Penicillium roqueforti, 559
Peroxidase, 308
importance, 308
inactivation by heat, 308
of bacteria, 310
Phosphatase, 301
inactivation by heat, 302
of bacteria, 304
preserving samples for, 304
reactivation, 303
Phosphates, *see* Washing reagents
Pipe line milkers, 147
quality of milk from, 148
rancid milk from, 149, 297
Plate count, 7
advantages, 8
Plate count, comparison with microscopic, 9
objections to, 8
Poisoning from dairy products, 282
Poliomyelitis virus, effect of heat, 207
from dairy products, 280
Preserving reagents, 168
antibiotics, 171
chlorine compounds, 170
formaldehyde, 169
general effect, 168
hydrogen peroxide, 169
Preserving samples for analysis, 172
Process cheese, spoilage, 585
Propionibacterium genus, 546
Propionibacterium peterssonii, 549
Propionibacterium shermanii, 546
Propionic acid, as mold inhibitor, 488, 533
Protease, 305
in cheese, 521
inactivation by heat, 306
of bacteria, 307
Proteolysis, 100
Proteolytic organisms, counting, 15
Pseudomonas aeruginosa, 168, 270
Pseudomonas cyanogenes, 105
Pseudomonas fluorescens, 102, 473, 501, 532
Pseudomonas fragi, 102, 246, 581, 583, 595
Pseudomonas graveolens, 104, 112, 596
Pseudomonas ichthyosmia, 323
Pseudomonas mephitica, 483, 595
Pseudomonas mucidolens, 104
Pseudomonas nigrifaciens, 491
Pseudomonas putrefaciens, 477, 595
media for, 477
Pseudomonas syncyanea, 105
Pseudomonas synxantha, 107
Pseudomonas viscosa, 581
Psychrophiles, 186, 246
counting, 19
defects produced, 187
heat resistance, 187
sources, 186

Q fever, effect on composition of milk, 264

Q fever, from milk, 263
role of livestock, 263
Quaternary ammonium compounds, *see* Bactericidal reagents

Rabbito, 475
Rabies from milk, 267
Rancidity, butter, 472
cheddar cheese, 529
milk, 28, 101, 295
pipe line milk, 149, 297
sweetened condensed milk, 335
Rayless goldenrod, 268
Red fermentation, 107
Reduction tests, 20
Resazurin test, 39
bases of reading, 40
comparison with methylene blue, 43
influence of penicillin, 44
influence of various factors, 41
Rhodotorula glutinis, 107
Richweed, 268
Roll tube technic, 22
Ropy fermentation, 82
causative organisms, 91
distribution, 97
heat resistance, 96
isolation, 97
sources, 94
controlling, 95
due to acid-forming species, 93
due to associative action, 93
materials responsible, 94
pasteurized milk, 96
Roquefort cheese, bacteriology, 558
Routine bacterial counts, 3, 4
significance on raw milk, 10
significance on pasteurized milk, 12

Salmonella organisms, effect of heat, 206
survival in milk, 284
Salmonella typhimurium, 283
Salmonella typhosa, effect of heat, 206
sources in milk, 275
Salt, in butter, 454, 473, 474
in cheeses, 523, 555, 560
Samples, collection and care, 23
Scarlet fever from dairy products, 279
Separation, 197, 492
Septic sore throat, from dairy products, 277
sources of causative organisms, 278
Serratia marcescens, 107, 121, 166, 473
Slimy fermentation, *see* Ropy fermentation
Small top pails, 128
Sorbic acid, as mold inhibitor, 534
Sour cream, cultured, 434
Spirit blue, to detect lipolysis, 18
Stable air, influence of factors, 124
organisms from, 123
measuring contamination, 123
reducing contamination, 125
Storage of milk in bottles and cans, 245
Streptococcus agalactiae, 11, 49, 57, 185, 269
destruction by chemicals, 270
Streptococcus citrophilus, 423
Streptococcus citrovorus, 376, 528
see Flavor organisms of lactic cultures
Streptococcus cremoris, 75, 402, 517
Streptococcus diacetilactis, 423
Streptococcus distendens, 323
Streptococcus durans, 341
Streptococcus equisimilis, 58, 269
Streptococcus faecalis, 537
Streptococcus lactis, 68, 183, 375, 522
associative action, 74
effect of rancidity, 399
fermenting capacity, 73
general characters, 71
growth conditions, 73
importance, 74
in lactic cultures, 375, 379
isolation, 75
reducing power, 35, 41
sources, 74
Streptococcus lactis var. *anoxyphilus,* 70
Streptococcus lactis var. *aromaticus,* 71
Streptococcus lactis var. *hollandicus,* 70, 93, 395
Streptococcus lactis var. *maltigenes,* 69, 93, 104
Streptococcus lactis var. *tardus,* 70
Streptococcus liquefaciens, 76, 100, 101, 122, 528
distribution, 77

Streptococcus liquefaciens, general characters, 77
isolation, 77
Streptococcus paracitrovorus, 376, 528
see Flavor organisms of lactic cultures
Streptococcus pyogenes, 185
Streptococcus thermophilus, 76, 341, 439, 544, 554, 557
Streptococcus uberis, 58, 269
Streptomycin, 171
Supervision of milk supplies, 249
essentials, 250
interpretation of counts, 251
Sweet-curdling fermentation, 98, 100
importance, 98
causative organisms, 99
Sweetened condensed milk, bacteriology, 327
examination, 327
spoilage, buttons, 332
chemical action, 334
control, 334
enzymatic action, 334
gas formation, 329
molds, 332
special defects, 334
thickening, 332
sugar concentration, 328
Swiss cheese, activity of cultures for, 545
bacteriology, 543
clarification of milk, 543
defects, 551
abnormal flavors and odors, 552
color defects, 553
gas, 551
lack of eyes, 551
eye formation in, 548
flavor contributors, 549
amino acids, 549
propionates, 549
influence copper and manganese, 550
lactobacilli in, 545
milk quality, 543
propionic acid organisms in, 546
protein decomposition, 550
S. thermophilus in, 544
Swiss-type cheese, 550

Tallowy flavor, butter, 480
milk, 29
sweetened condensed milk, 335
Temperature, influence on milk fermentation, 175
Tests, for general quality of milk, 26
for products of growth in milk, 26
for rate of changes in milk, 34
for special organisms in milk, 44
Thermoduric organisms, detection, 217
effect of cooling, 216
effect of homogenization, 217
in pasteurization, 213
resistance to chemicals, 218
significance, 218
sources, 214
Thermophilic organisms, counting, 19
in pasteurization, 212
Torula amara, 528
Torula cremoris, 83
Torula sphaerica, 83
Torulopsis globosa, 331
Torulopsis lactis-condensi, 330
Torulopsis sphaerica, 83
isolation, 85
Trembles, *see* Milk sickness
Tremetol, 268
Tributyrin, to detect lipolysis, 18
Trimethylamine, in fishy butter, 474
Tripropionin, to detect lipolysis, 18
Tuberculosis, bovine organism in human disease, 255
control from dairy products, 258
introduction into herd, 258
organism, *see Mycobacterium tuberculosis*
spread among cows, 258
Typhoid fever, from cheese, 516
from dairy products, 274
organism, *see Salmonella typhosa*

Udder, clipping, 128
organisms in, 117
Udder condition, influence on count, 11, 118
Udder infections, tests for, 49
acidity, 50
appearance, 50
body cells, 51

Udder infections, bromthymol blue, 50
catalase, 50
chlorides, 51
curd tension, 59
Hotis, 57
lactose, 51
physical examination, 49
streptococci, 56
Whiteside, 58
Ultraviolet rays, effect on bacteria, 229
effect on molds, 535
Ultrasonic waves, effect on bacteria, 230
Unclean flavors, butter, 483
cheese, 529
Undulant fever, *see* Brucellosis
Utensils, *see* Equipment

Vacuum lines, care, 147
contamination from, 140
Vats, *see* Equipment
Vibrio comma, effect of heat, 206
survival in milk, 284
Vitamins, effect of pasteurization, 225
in cheeses, 591
production, 591
retention, 591
Volatile acids, formed by *S. lactis*, 73
in cheddar cheese, 524
in lactic culture, 380

Washing reagents, 156
acids, 158
bactericidal action, 160
alkalies, 157
bactericidal action, 158
Washing reagents, chelating agents, 158
phosphates, 157
wetting agents, 157
Water, for cooling, 176, 493
Water storage tanks, 598
Water supplies, bacteriology, 593
detection spoilage organisms, 599
general studies, 597
source of spoilage organisms, butter, 594
cheese, 596
standards, 600
Water supplies on farms, 136
Water-insoluble acids, in butter, 500
factors influencing, 501
in cream, 499
relation to fat acidity, 502
Wetting agents, *see* Washing reagents
Whey, expulsion from cheese, 517
White snakeroot, 268
Whiteside test, 58
Wisconsin curd test, 45

Yeast and mold standards, butter, 447
Yeasts, counting, 19, 445
in cheddar cheese, 528, 530
in cottage cheese, 581
in cream, 83
in milk, 108
on brick cheese, 556
on limburger cheese, 570
Yeasty flavor, butter, 470
Yellow fermentation, 106
Yogurt, 438
organisms in, 439

637.H22D 1957

a39001 007473187b

67-11
69-1
70-1
73-1